Universidade de Aveiro Departamento de Biologia
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Marta Martins Alexandre

Environmental drivers of mesocarnivores presence in Mediterranean landscapes

Condicionantes ambientais à presença de mesocarnívoros em paisagens mediterrânicas

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor Luís Miguel do Carmo Rosalino, Investigador Auxiliar do Departamento de Biologia e do Centro de Estudos do Ambiente e do Mar (CESAM) da Universidade de Aveiro, com coorientação do Doutor Eduardo Manuel Silva Loureiro Alves Ferreira, Professor Auxiliar Convidado do Departamento de Biologia da Universidade de Aveiro.

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palavras-chave

Amostragem não-invasiva; Carnívoros; Constrangimentos ambientais; Dejetos; Distribuição; Heterogeneidade espacial; Identificação molecular; Identificação morfológica; Monitorização.

resumo

Os ecossistemas terrestes apresentam uma elevada complexidade e, as espécies que neles habitam, incluindo o Homem, interagem entre si influenciando a forma como cada uma explora e utiliza os recursos disponíveis. Com o crescimento da população humana e, consequentemente, das áreas urbanas e de terrenos dedicados à produção de bens para consumo humano, as paisagens têm vindo a sofrer alterações, conduzindo a uma acelerada perda de habitat, sendo esta considerada a principal causa do declínio da biodiversidade. Devido a este declínio, as espécies enfrentam desafios à sua sobrevivência, moldando a sua ecologia como forma de se adaptarem às novas condições ambientais, tendo estas adaptações repercussão em todo o ecossistema. Os carnívoros, estando em elevados níveis tróficos, desempenham um importante papel na estrutura e no bom funcionamento dos ecossistemas, sendo essencial o desenvolvimento e implementação de planos de conservação efetivos para a preservação destas espécies. Devido a serem um grupo que, na sua maioria, possui hábitos noturnos e/ ou crepusculares, apresenta elevada mobilidade e reduzidas densidades, os carnívoros são normalmente monitorizados através do estudo dos seus indícios de presença, em particular, dos dejetos, por estes serem abundantes e fáceis de encontrar. Até recentemente os dejetos eram identificados, exclusivamente, através de critérios morfológicos e odoríferos. No entanto, devido à elevada incerteza associada a esta técnica, novos métodos de amostragem não-invasiva usando técnicas moleculares começaram a ser utilizados, revelando-se uma solução eficaz e precisa para uma identificação mais rigorosa. O presente estudo teve como principais objetivos: 1) testar a precisão da identificação de dejetos de uma comunidade de mesocarnívoros na região Nordeste de Portugal, através da aplicação do método convencional (critérios morfológicos e odoríferos), utilizando como critério de precisão o resultado da identificação molecular; e 2) perceber, com recurso à modelação ecológica (GLMM), de que forma o contexto paisagístico influencia a presença de duas espécies generalistas – a raposa (*Vulpes vulpes*) e a fuinha (*Martes foina*) – numa área fortemente marcada pela presença de áreas naturais e pela prática da agricultura tradicional. No total, entre julho e setembro de 2016, foram registados 291 indícios de presença de mesocarnívoros (dejetos e pegadas). Desse conjunto, 63 dejetos foram analisados geneticamente. Destes foi amplificado e sequenciado, com sucesso, o ADN de 83% das amostras. A taxa de sucesso mais elevada na classificação das espécies, com base em critérios morfológicos, foi de 67%, sendo que o sucesso da identificação variou de espécie para espécie. Dos 291 indícios registados e, tendo por base a classificação do observador com a maior taxa de sucesso, conclui-se que 212 indícios pertenciam a raposa e 55 a fuinha. Os modelos de distribuição gerados revelaram que a raposa aparenta ter duas estratégias distintas. Quando em ambientes com elevada percentagem florestal, as atividades humanas exercem um efeito negativo (causam perturbação). Já quando a percentagem florestal é reduzida, a presença desta espécie é influenciada positivamente pela atividade agrícola e a proximidade à área urbana (disponibilidade de alimento). Em relação à fuinha, a sua distribuição é afetada negativamente por atividades de origem antropogénica (alteração e perturbação do habitat), contudo a proximidade a meios urbanos exerce um efeito positivo (permite a obtenção de alimento e evita a competição com outros mesocarnívoros). Os nossos resultados suportam a necessidade do uso da identificação genética como ferramenta em estudos de ecologia de carnívoros, pois conferem um maior grau de certeza e rigor à identificação específica. Conseguimos ainda confirmar, o caracter oportunista e a capacidade de adaptação da raposa e da fuinha a ambientes antrópicos. Este estudo contribui para um melhor conhecimento da dinâmica e estratégias de alguns dos mesocarnívoros mais comuns em Portugal, crucial para a elaboração e aplicação de ações de conservação e gestão destas espécies, uma vez que demonstrámos que, pelo menos para a raposa, o contexto paisagístico influencia o padrão de uso do espaço detetado. Este facto sugere que considerar que diferentes populações de uma espécie são condicionadas pelos mesmos fatores, independentemente do contexto paisagístico, tornará ineficientes as medidas de gestão delineadas para assegurar a sobrevivência regional das espécies-alvo.

keywords

Carnivores; Distribution; Environmental constrains; Molecular identification; Morphological identification; Monitoring; Non-invasive sampling; Scats; Spatial heterogeneity.

abstract

Terrestrial ecosystems are highly complex and the species inhabiting them, including Humans, interact with each other influencing how each one exploits the available resources. With the growth of the human population and, consequently, of the urban areas and those devoted to produce goods for humans, the landscapes have undergone changes leading to an accelerated loss of habitat, which is considered the main cause for biodiversity decline. Due to this decline, species face challenges to their survival, shaping their ecology to adapt to new environmental conditions, and these adaptations have cascading repercussions throughout the ecosystem. Carnivores, being at high trophic levels, play an important role in the structure and proper functioning of ecosystems. The development and implementation of effective conservation plans is essential for the preservation of these species. Since they are mostly nocturnal and / or crepuscular, have high mobility and low densities, carnivores are usually monitored through the study of their signs of presence, in particular, their scats, because these are abundant and easy to find. In earlier studies, scats were exclusively identified through morphological and odoriferous criteria. However, due to the high uncertainty associated with this technique, new non-invasive sampling methods using molecular techniques began to be used, proving to be a solution for a more rigorous and accurate identification. The present study aimed: 1) to test the accuracy of mesocarnivores scats identification, from a community in the Northeastern region of Portugal, based on a conventional approach (morphological and odoriferous criteria), using as a standard for accuracy the results of molecular identification; 2) to understand, using the ecological modelling approach (GLMM), how the landscape context influences the presence of two generalist species - the red fox (*Vulpes vulpes*) and the stone marten (*Martes foina*) - in an area composed by natural areas and the practice of traditional agriculture. In total, between July and September of 2016, 291 mesocarnivores' signs of presence (scats and footprints) were recorded. From this sampling, 63 scats were genetically analysed and the DNA of 83% of the samples was successfully amplified and sequenced. The highest success rate in species identification, based on morphological criteria, was 67%, but the success of the identification varied among species. Of the 291 recorded data and based on the observer's classification with the highest success rate, 212 signs of presence were considered to belong to the red fox and 55 to the stone marten. The generated distribution models revealed that the red fox appears to have two distinct strategies. When inhabiting an environment with a high percentage of forest, human activities have a negative effect (disturbance in habitat). Inversely, when in an environment where the forest cover is reduced, the presence of this species is positively influenced by the agricultural activity and the proximity to urban area (food availability). Stone marten's distribution is negatively affected by anthropogenic activities (habitat alteration and disturbance), although the proximity to urban has a positive effect (allows to obtain food and avoids competition with other mesocarnivores). Our results support the need for the use of genetic identification as a tool in carnivore ecology studies, since it allows for a higher accuracy and provide more rigor to the morphological identification. We were also able to confirm the opportunistic nature and adaptability of the red fox and the stone marten. This study contributes to improve our knowledge of the dynamics and strategies of some of the most common mesocarnivores in Portugal, crucial for the application of conservation and management actions focused on those species. Furthermore, we demonstrated that, at least for the red fox, the landscape context influences the pattern of distribution. Consequently, by considering that populations of the same species are constrained by the same factors, independently of the landscape composition, will affect the efficacy of management measures put in place to assure the regional survival of a species

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Publications

National Congress

Alexandre, M., Rosalino, L. M., Hipólito, D., Fonseca, C., Ferreira, E., 2017. "Can scats be a misleading ecological tool? More evidences from northeastern Portugal", 16ºEncontro Nacional de Ecologia – SPECO, Reitoria da Universidade de Lisboa, 9-10 novembro 2017. Portugal. (Poster)

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Chapter I

1. General Introduction

Over the last century, there has been an increase of the human population worldwide (United Nations, 2014). Consequently, natural habitats have been seriously reduced and fragmented, which significantly threatens local and global biodiversity (Turner et al., 2004). About 83% of the earth's surface is affected by anthropogenic activities (Fahrig, 2003) and habitat transformations – the alteration of natural ecosystems and/ or the creation of new anthropic environments – has consequences on the wildlife species that depend on these to survive (Hoekstra et al., 2004).

According to the United Nations, habitat loss is considered to be the leading cause of biodiversity's global decline (Millenium Ecosystem Assessment, 2006). This loss doesn't consist exclusively of habitats destruction; it can also be a consequence of habitats conversion into another land uses, such as agricultural systems (FAO, 2012; Geist and Lambin, 2002), that ensure the subsistence and well-being of the human species (Millenium Ecosystem Assessment, 2006). An example of a long-lasting habitat change, due to the combined action of various human activities, can be seen in the landscapes of the Mediterranean region. Historically dominated by forests, these landscapes began to undergo transformations in the Neolithic period, when the domestication of livestock and the use of land as agricultural production areas began (Barbero et al., 1990; FAO, 2012). The intensification of these activities over time, led to the deforestation of several areas throughout Mediterranean Europe (Barbero et al., 1990; Blondel, 2006; FAO, 2012).

Changes that occur in a single habitat can create a chain of events that may affect the entire ecosystem (Pereira et al., 2012). Thus, the individual and/ or combined action of anthropic factors, can cause the fragmentation of populations both in terms of their distribution and abundance, as well as in their genetic diversity (Blondel, 2006). Despite the disturbances to which the Mediterranean habitats have gone through and continue to be subject to, the number of species present in the region is considered high, when compared with other areas (Myers et al., 2000). Carnivores are among the species that may be affected by transformations in the landscapes (Dobrovolski et al., 2013), but generalist species have been able to resist over the times, due to their adaptability to explore the novel available resources in the new landscapes (e.g. olive and wheat consumption by badgers in Portugal – Hipólito et al., 2016; Rosalino et al., 2005; free-ranging poultry predation by foxes and mustelids in a French rural area – Stahl et al., 2002). Furthermore, species from this taxa are particularly important because they have an central role in the ecosystem functioning (Gittleman, 1989) – e.g. prey populations controllers, etc. (Norrdahl and

Korpimäki, 2000; Rosalino et al., 2010) –, and changes in their guild composition have cascading effects upon all community.

1.1. Mesocarnivore's ecology - General patterns

Carnivores are distributed all over the world (with the exception of Antarctica), occupying different ecological niches (Hunter, 2011) and presenting a large interspecific variety, namely in terms of body size, behavioural habits and strategies of life (Hunter, 2011; Loureiro et al., 2012). Despite this diversity, carnivores are in fact a homogeneous group determined by their main characteristic – they are mostly predators (Ruiz-Olmo, 2012), with only very few species shifting their feeding behaviour towards a more herbivore diet (e.g. giant panda, *Ailuropoda melanoleuca*; Reid et al., 1989). Nevertheless, the amount of animal food in their diet varies between species (felids are highly carnivorous while some mustelids or procyonids are mostly omnivores; Hunter, 2011), or even between populations of the same species (ex. red fox; Díaz-Ruiz et al., 2013).

The order Carnivora is formed mostly by species that have small to medium-sized dimensions (weight between 1 and 15kg), called mesocarnivores (Prugh et al., 2009; Ruiz-Olmo, 2012). Due to their smaller size, energetic demands, feeding behaviour and their ability to thrive in the most diverse habitats, they are generally more abundant than species with larger dimensions (Roemer et al., 2009). The ecological importance of these mesocarnivores in ecosystems is unquestionable. They are key species in the regulation of ecosystems (Gittleman, 1989), and in maintaining the structure of the biological community in which they are inserted, playing crucial functional roles – regulation of lower trophic levels (Roemer et al., 2009); support of the resilience of ecosystems (Ritchie et al., 2012); disease control (Roemer et al., 2009); and seed dispersal (Rosalino et al., 2010) –, so their preservation ensures that many other species, that share the same niche, can also survive (umbrella species; Mangas et al., 2008). Therefore, mesocarnivores are often a target group for the application of landscape monitoring and management programs (Barea-Azcón et al., 2007a), being frequently used in conservation by proxy approaches (Caro, 2010).

However, this important role may be in risk. These species are increasingly being threatened by direct and indirect impacts associated with man and his actions on the landscape. The fact that they are predators of wild species with commercial interest, create conflicts with human populations (Treves and Karanth, 2003; often remedied through illegal hunting). Furthermore, some species of mesocarnivores can be legally hunted as they are considered game species in some regions (e.g. red fox, *Vulpes vulpes*, and Egyptian

mongoose, *Herpestes ichneumon*, in Portugal; Loureiro et al., 2012; Pereira et al., 2012). The threats are also related to changes in the landscape composition (e.g. conversion of natural environments into agriculture lands; Blondel, 2006; Loureiro et al., 2012) and anthropic disturbance (e.g. livestock and road network; Galantinho and Mira, 2009; Grilo et al., 2009), that may bring serious consequences to the ecology and survival of mesocarnivores' populations (e.g. Dobrovolski et al., 2013; Silva et al., 2017). Furthermore, as Mediterranean ecosystems are inherently heterogeneous due to a legacy of human alterations to the landscape and climate variability (Blondel, 2006), understanding how both factors (i.e. anthropic disturbance and landscape composition), such as altitude (influence the temperature and the terrain slope), affect carnivores' presence pattern, is an important ecology goal (Manel et al., 2001; Pereira et al., 2012; Silva et al., 2013).

In Portugal, the carnivores' guild is diverse and composed by 15 species (Álvares et al., 2017). Distinct species – specialists and carnivorous (such as many felids) to generalist and ubiquitous species (e.g., medium size canids) – have different habitat use patterns, so they will response, in a different way, to the changes in the landscape composition (Morrison et al., 2012; Nowark, 2005). Thus, is essential to understand how carnivores respond to altered environments in the Mediterranean heterogeneous landscapes.

1.2. Monitoring methods

Correct species identification is a key factor in conservation biology and is the basis for any study dedicated to wildlife conservation (Carreras-Duro et al., 2016; Harrington et al., 2010). As mentioned previously, the preservation of carnivores is important for the proper functioning of ecosystems and the accuracy in determining species distribution, within the area of interest, is the first step for conservation program (Carreras-Duro et al., 2016; Rodríguez et al., 2007). Most of the monitoring protocols are constrained by time and budget limitations (Barea-Azcón et al., 2007a). Therefore, these factors must be considered prior to implementing the sampling strategy.

Mammalian carnivore data collection is especially challenging because they are mostly nocturnal and/ or crepuscular and, frequently, have large distribution areas (Gese, 2001; Wilson and Delahay, 2001), which hinders their direct observation. Thus, non-invasive methods based on indirect signs of presence (e.g. footprints, faeces and shelter structures) are being increasingly used in monitoring studies of species presence and habitat use patterns (Gompper et al., 2006; Kelly et al., 2012; Sutherland, 2006). The use of dirt roads or pedestrian paths (i.e. linear transects) to search for species sign of presence,

is one of the most recurrently used methods to carry out studies on the distribution of carnivores. Among all these signs of presence, scats is one of the most informative, due to the abundance and conspicuousness of the faeces (Waits and Paetkau, 2005). Besides its conspicuousness, scats also allow to study different aspects of mesocarnivores' ecology. For example, in many countries, the identification of scats found along the transects, is used to monitor the populations of stone marten (*Martes foina*; Virgós et al., 2012) and red fox (Sadlier et al., 2004), among other species. In addition, scats also allow to study the distribution of the species (Bonesi et al., 2006), as well as assess it diet composition (Hipólito et al., 2016; Rosalino et al., 2005) and unravel possible intraspecific (Bianchi et al., 2014) and interspecific competition between sympatric species (Brzeziński et al., 2008; Remonti et al., 2012). However, this method is not infallible and its application can introduce a bias into the analysis that need to be acknowledged and dealt with, so results can be properly interpreted.

There may be an interspecific detectability variation in the signs of presence during the pedestrian transects, which is related to species abundance and marking behaviour (Harington et al., 2010), habitat type or even researcher' skills and field experience. Thus, it is necessary to consider this factor when defining the sampling protocol and when selecting analytical approach to the data (Boulinier et al., 1998). Other limitation of scat surveys, is related with the high error rates that occur in their identification. This last error has been reported as a recurring problem (Birks et al., 2004; Davison et al., 2002), which has even greater impact when the faeces are produced by sympatric and similar sized-species (e.g. red fox, stone marten and pine marten, *Martes martes*; Laguardia et al., 2015), since they may share the same food resources, producing highly similar scats (Foran et al., 1997).

1.2.1. The use of molecular identification in ecological studies

Accurate knowledge of species distribution is extremely important in the design of wildlife conservation plans. The strategies based on data where scats identification is inaccurate, can lead to a misdirection of management efforts and undermine the effectiveness of species protection programs (Harrington et al., 2010). Therefore, overcoming the mentioned problems through non-invasive methods, has become a priority for ecologists.

The development of molecular technologies has allowed to improve the precision and the confidence in the use of the non-invasive methods (Piggott and Taylor, 2003), proving these to be an opportunity to move forward in understanding the species and

populations ecological adaptations to different landscape contexts (Monterroso et al., 2016, 2013; Palomares et al., 2002), avoiding unnecessary use of time and resources (Harrington et al., 2010). Since the Polymerase Chain Reaction (PCR) amplification of DNA (**D**eoxyribo**n**ucleic **A**cid) sequences was discovered by Kary Mullis (Arnheim et al., 1990; Morin and Woodruff, 1996), this technique has been applied in scientific fields such as development, ecology, evolution, behaviour and conservation (Fernando et al., 2003). The PCR technique has made the study of the genetic material, present in scats, a feasible approach, by making it possible to isolate, amplify and analyse specific DNA fragments from low-quantity and low-quality DNA, thus enabling vital information regarding populations to be obtained, such as species, individuals or gender identification, pathologies, feeding habits, population size and kinship relationships (Kohn et al., 1995; Kohn and Wayne, 1997).

When using molecular techniques, it is necessary to take into account multiple factors that, both in the field and in the laboratory, can contribute to the quality and quantity of the DNA present in the faeces (Murphy et al., 2007). For example, sampled scats should be collected in the field using sterile instruments (Foran et al., 1997), in order to reduce the risk of contamination. It should also be a priority to collect samples as fresh as possible, as it increases the probability of successful identification by molecular methods (Taberlet et al., 1996; Taberlet and Luikart, 1999). Another problem with DNA analysis is the cost of some procedures (e.g. microsatellite analysis) compared to the morphological identification, which can be one of the reasons for not using the molecular methodology. Therefore, it is necessary to balance the costs of the molecular techniques used in our work, against all risks and future implications of the non-verification of the field signs identity (Harrington et al., 2010).

Nonetheless, molecular techniques have become an essential tool in research, allowing to ensure reliable data collection, increase the accuracy in species scats identification (solving the errors that occur in the morphological identification) and, consequently, in assessing carnivores' ecology.

1.3. Objectives

Considering all the ecological context and constraints previously mentioned, this study intended to contribute not only to the understanding of how mesocarnivores are influenced by regional landscapes and anthropic characteristics, which may affect how populations are managed; but also, to provide practical information that can be used in the conservation of

some mesocarnivores species, inhabiting Mediterranean habitats, by showing the limitations of methods often used to study this taxon. So, the main objectives of this work are: 1) test the accuracy of mesocarnivore's scats identification based on odor and morphology (conventional approach), using as a standard of accuracy the results of molecular identification of the scats (molecular approach); and 2) understand how the landscape context affects the presence of red fox (*Vulpes Vulpes*) and stone marten (*Martes foina*), by generating distribution models to test different ecological hypothesis. In order to respond to the last goal, we formulated three different ecological hypotheses based on the variables that, based on the available ecological information for each species (see Chapter IV), may influence the presence of red fox and stone marten: i) disturbance hypothesis, when the presence of species is influenced by anthropic disturbance factors; ii) altitude hypothesis, when the altitude is the main factor determining the species distribution; and iii) landcover composition hypothesis, when the most determinant variables are related to the landscape composition.

In face of all the data gathered, this work will contribute with key aspects on the ecology of these two species, which might be useful to create more efficient monitoring and conservation programs.

Chapter II

2. Study Area

The study was implemented in Northeast Portugal (41º30'33.3"N, 6º56'57.5"W) covering the districts of Bragança and Vila Real (Figure II.1), two of the eighteen districts of mainland Portugal. The district of Bragança is divided into twelve municipalities, with a low population density (19,6 ind/Km^2, varying between 9,7 ind/Km^2 in Vimioso and 36,2 ind/Km^2 in Mirandela) (PORDATA, 2011) and a resident population of 136 252 inhabitants (INE, 2011a). Vila Real district is subdivided into fourteen municipalities with a total resident population of 206 661 inhabitants (INE, 2011b) and an overall density of 70 ind/Km^2, ranging from 13,1 ind/Km^2 in Montalegre to 180,6 ind/Km^2 in Peso da Régua (PORDATA, 2011).

The study area (Figure II.1) encompasses the entire range of the district of Bragança (6,608 Km^2 area) and five of the 14 municipalities of Vila Real (Alijó, Chaves, Murça,

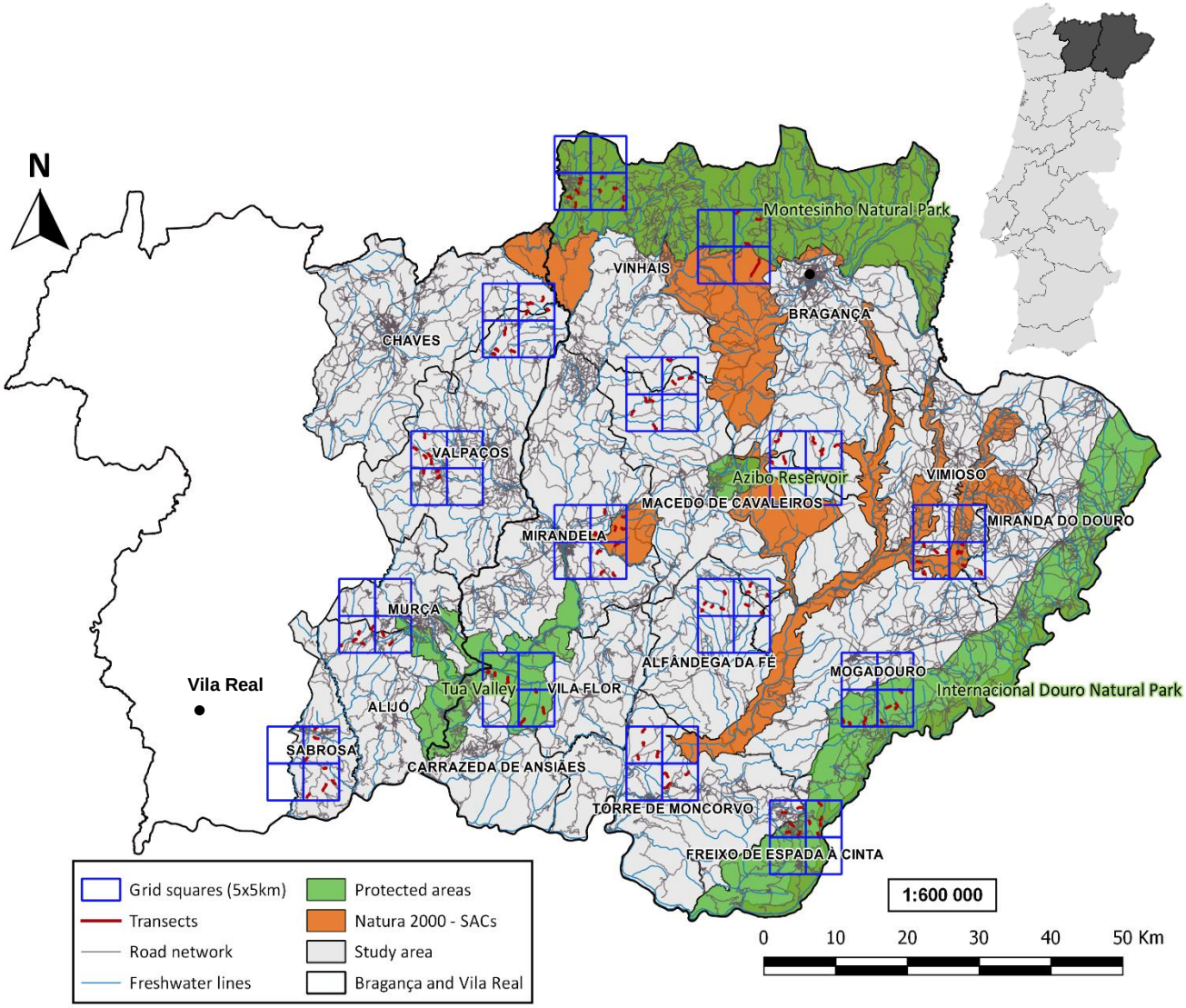

Figure II.1 – The study area location in the districts of Bragança and Vila Real (region of Trás-os-Montes), highlighting the sampled municipalities and the spatial distribution of the sampling sites (represented by the grid cells and transects).

Sabrosa and Valpaços – 1,787 Km^2 area), covering a total of 8,395 Km^2, bounded by the river Douro at the South, the Alvão-Marão mountain at the West and the Spanish border to the North and East. Both districts constitute, according to the previous administrative classification of Portugal in provinces, the region of Trás-os-Montes, located on the northeastern corner of the country (Fleskens et al., 2009), but whose limits have been changing throughout history. After the implementation of the hierarchical system of division of territory for statistical purposes – Nomenclature of Territorial Units for Statistics (NUTS) – in 1989, a new regional division was established in Portugal (Ministério do Planeamento e da Administração do Território, 1989). The province of Trás-os-Montes is now divided into three administrative NUTS: Alto Tâmega (PT11B), Douro (PT11D) and Terras de Trás-os-Montes (PT11E) (European Commission, 2014; PORDATA, 2017), all overlapping our study area.

In the region, there are three major orographic units that mould the landscape: the main mountains – Alvão and Marão (with a maximum altitude of 1,283 m and 1,415 m, respectively), Montesinho (1,486 m), Nogueira (1,319 m) and the Morais Massif (altitudes ranging from 300 to 900 m); a transition plain area between the mountain and the valley; and the warm valleys with lower altitudes, but with steep slopes, where vineyard, olives and other crops are planted (Everpidou et al., 2010). The climate is strongly influenced by these orographic characteristics (Costa et al., 1998), mainly the massive system of mountains. The climate is Mediterranean, but it has Atlantic and Continental influences due to its location in a transitional zone between the Atlantic climatic area of northern Iberia and the continental region of central Iberian Peninsula. Furthermore, the Alvão-Marão cordillera blocks the clouds and winds coming from the sea, creating a condensed and humid environment. These conditions result in an annual variation characterized by long and cold winters and hot and dry summers (Everpidou et al., 2010). In Bragança, the average temperature varies between 4,5ºC in the coldest month (i.e. January) and 21,7ºC in the warmest month (July), and the average annual precipitation is 770mm (IPMA, 2017a). In the district of Vila Real the average temperature varies between 6,3ºC in the coldest month (January) and 21,7ºC in the warmest month (August). The average annual precipitation is higher, reaching 1020mm (IPMA, 2017b).

The local flora is diverse and the landscape composition varies, being possible to find different land covers along the study area – deciduous forests (*Castanea sativa*, *Quercus pyrenaica*, *Quercus rotundifolia*, *Quercus suber*), conifer forests (*Juniperus oxycedrus, Pinus pinaster, Pinus sylvestris, Pseudotsuga menziesii*), scrublands (*Cistus ladanifer, Cytisus spp., Erica tetralix*, *Genista anglica)* and permanent natural meadows

(*Festuca indigesta*, *Oxalis pes-caprae*; Azevedo, 2012; ICNF, 2017a), among others. In addition to the natural habitats, the landscape is also characterized by extensive and traditional agricultural activities (production of almond, chestnut, olive and vineyard) and by grazing systems (e.g. "Maronesa" and "Mirandesa" autochthonous cattle breeds) (Everpidou et al., 2010; Madureira et al., 1994; Oliveira Baptista et al., 2003).

Trás-os-Montes is one of the Portuguese regions that hosts higher vertebrate biodiversity values, particularly of carnivores, hosting 13 of the 15 carnivores inhabiting Portugal (Álvares et al., 2017). Furthermore, some of those species are endangered, enhancing the national importance of this region for the Iberian carnivore community. For example, Vila Real and Bragança districts, together, constitute one of the most important areas for the conservation of Iberian wolf (*Canis lupus signatus*; ICNF, 2017b), an Iberian top predator, classified as "Endangered" at the national level (Cabral et al., 2005). The region is also one of the few areas, at national level, where the presence of ermine (*Mustela erminea*) and pine marten (*Martes martes*), both classified as "Data Deficient" by the Portuguese Red List Book (Cabral et al., 2005), was confirmed (Matos, 2012; Sales-Luís, 2012). Other more common carnivores, such as the stone marten (*Martes foina*), the European badger (*Meles meles*), the red fox (*Vulpes vulpes*), or the introduced species – common genet (*Genetta genetta*) – are also present (Álvares et al., 2017). In Northeast Portugal, wild ungulates still live in sympatry, reinforcing the importance of this area for mammalian conservation. In the region, wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and deer (*Cervus elaphus*) population coexist with domestic herbivores, such as regional breeds of cattle, goats and sheep (Everpidou et al., 2010).

Due to this flora and wildlife values several areas, within this region, integrate the Portuguese National System of Classified Areas (Sistema Nacional de Áreas Classificadas – SNAC). Some, such as the Montesinho Natural Park and the International Douro Natural Park (Figure II.1), are part of the National Network of Protected Areas (Rede Nacional de Áreas Protegidas – RNAP) and are classified under a legal protection status that aims the adequate protection and maintenance of biodiversity, while providing services for ecosystem that maintain the natural and geological heritage (Azevedo, 2012; ICNF, 2017c; Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional, 2008). Other areas have a special status at international level, since they are part of the European Union's Natura 2000 Network, within the Special Areas of Conservation (SACs) category, under the Habitats Directive – e.g. PTZPE0037 Rios Sabor e Maçãs (Natura 2000, 2017a) and PTCON0043 Romeu (Natura 2000, 2017b).

Chapter III

This chapter was submitted to ***Mammalian Biology*** (formerly *Zeitschrift für Säugetierkunde*), as a "Short note".

3. "Can scats be a misleading ecological tool? More evidences from northeastern Portugal"

3.1. Abstract

Species identification of non-invasively collected samples using molecular genetics tools has become an important instrument in ecological research and conservation studies. This approach allows the study of wildlife without the need of capture or observe the animals *in situ*, which is especially important for carnivores. Furthermore, their elusive behaviour makes carnivore species difficult to study and an adequate, efficient and accurate monitoring is essential for an effective conservation. For decades, scat-based ecological studies were solely rooted on morphological and odoriferous identification of scats. However, the use of this approach has raised issues and originated a controversial debate, due to the high probability of error and the lack of validation. Due to technological development, new methods of non-invasive monitoring of animal populations, using genetic markers, became available and cost-efficient in the last decade, being relevant to overcome the referred problems. The objective of this study was to test the accuracy of mesocarnivores scats identification, based on a conventional approach (morphological and odoriferous criteria), using as a standard for accuracy the results of molecular identification. We extracted DNA from 63 faecal samples of sympatric mesocarnivores, from a northeastern region of Portugal, and amplified fragments of the D-loop of the mitochondrial DNA (mtDNA) control region. DNA was successfully amplified and sequenced from 83% (*n*=52) of the extracts. Samples were assigned to red fox (*Vulpes vulpes*, *n*=38), stone marten (*Martes foina*, *n*=8), pine marten (*Martes martes*, *n*=2), European badger (*Meles meles*, *n*=1), common genet (*Genetta genetta*, *n*=1) and domestic dog (*Canis lupus familiaris*, *n*=2). Error rate in species assignment of scats (% of times a scat is miss assigned) based on morphological criteria was highly variable, ranging from 4% for red fox samples to 100% for some species, such as the European wildcat (*Felis silvestris*). The rate at which a scat of a given species was assigned to other species was also highly variable, ranging from 0% (common genet and European badger), 25% or 29% (in stone marten and red fox) to 100% (pine marten and Iberian wolf, *Canis lupus familiaris).* The results support the need to implement molecular methods in ecological studies based on scat identification, so that researchers can determine the error rates associated with morphological discrimination, and incorporate the user and species associated accuracy variation in the development of accurate monitoring studies.

Keywords

Mesocarnivores – Mitochondrial DNA – Monitoring – Non-invasive sampling – Species molecular identification

3.2. Introduction

Surveying wild species in the wilderness is a challenging and often time-consuming and expensive activity, depending on the species or species groups considered (Davison et al., 2002). Mammalian carnivore data collection is especially challenging because they are mostly nocturnal and/ or crepuscular, have high mobility, often occupy large home ranges, present low densities and are frequently sensitive to disturbance (Gese, 2001; Wilson and Delahay, 2001).

Carnivores play a key role in the structure and functioning of ecosystems (Gittleman, 1989) and a rigorous and accurate monitoring is fundamental to the development and implementation of adequate and effective conservation plans (Kelly et al., 2012). For this reason, the accuracy and reliability of data collection are decisive (Heinemeyer et al., 2008). Furthermore, carnivores' elusive behavior, body size and conservation status imposes an additional difficulty in implementing methods that involve the capture and/ or handling individuals, which may become stressful and potentially dangerous for handlers and wildlife (Kelly et al., 2012; Morin and Woodruff, 1996; Taberlet and Luikart, 1999). As a result, information about these animals depends on non-invasive sampling, specifically on indirect evidences of the presence of a species (scats, footprints, claw-marking; Gompper et al., 2006; Kelly et al., 2012; Waits and Paetkau, 2005). Among these methods, scats' identification is one of the most informative and frequently used methods for the detection and monitoring of small and medium-sized carnivores (mesocarnivores) in Europe (Barea-Azcón et al., 2007; Davison et al., 2002; Rosellini et al., 2008). This is due to scat abundance and conspicuousness, and also the diversity of information that can be obtained from it (e.g. diet, parasite burden, species-habitat associations; Putman, 1984).

However, the information obtained from mesocarnivores' scats can only be useful if based on correct species identification. Success rate estimation becomes even more relevant when the faeces are produced by sympatric and similar size-species – e.g. red fox (*Vulpes vulpes*), stone marten (*Martes foina*) and pine marten (*Martes martes*; Laguardia et al., 2015) – that may share the same food resources (Foran et al., 1997) and produce highly similar scats, that are often deposited in similar structures (e.g. along dirt roads). High identification error rates are reported as a recurring problem (Birks et al., 2004; Davison et

al., 2002), and to overcome these problem, non-invasive molecular methods have been applied (Kohn and Wayne, 1997). These methods allow accurate species identification by amplifying and sequencing a small mitochondrial (mtDNA) or nuclear DNA (nDNA) fragment (Beja-Pereira et al., 2009; Palomares et al., 2002).

Based on the considerations mentioned above, the main objective of this study is to test the accuracy of mesocarnivores' scat identification based on a conventional approach (morphological and odoriferous criteria), using a Mediterranean mesocarnivore guild as model. As a standard, for accuracy, we will use the results of scats' molecular identification. By providing identification error estimates for each species, we expect to provide relevant insights for carnivore ecological studies, but also, practical information for the conservation of mesocarnivores species in Mediterranean habitats.

3.3. Material and methods

3.3.1. Field sampling

The study was implemented in Northeast Portugal (41º30'33.3" N, 6º56'57.5" W) – Bragança and Vila Real districts – covering a total area of 8 395 Km^2 (Figure II.1). Samples were collected in summer (July–September, 2016), when the offspring of most mesocarnivores begins to be more independent (Loureiro et al., 2012). During this period, we monitored a total of 143 transects (500m long) distributed throughout a 5x5km square grid (4 or 5 transects per grid cell), to adequately sample all existing habitats (Figure II.1). Transects were located along trails or dirt roads, surveyed on foot by one or two observers to search for mesocarnivores' signs of presence (scats and footprints).

All the scats were initially identified in the field based on their location, morphology, odour and dimensions with the support of field guides (Bang and Dahlstrom, 2006; Sanz, 2003), and later by other observers, through the analysis of photographic records. Four observers with two different levels of experience in scats' morphological identification (i.e. experienced – several years of field experience and not experienced researchers), identified all the mesocarnivores' scats, based on the photographs of scats and surrounding area.

A total of 96 scat samples were carefully collected, with disposable sterile gloves, to avoid contamination, and stored in plastic containers (identified with the sample code) in 96% ethanol, until DNA extraction. Attempts were made to collect the most recent samples (nearly intact and moist), since it increases the probability of success in the identification by molecular methods (Foran et al., 1997; Taberlet et al., 1996). We also targeted samples with different morphology, in order to increase the possibility of belonging to different

species (Bang and Dahlstrom, 2006). Collected samples were carried on a cooler bag with ice packs to maintain the temperature low and prevent sample degradation, until arriving in the laboratory, where were conserved in a refrigerated environment (-20ºC).

3.3.2. Laboratory procedures - Molecular identification

DNA isolation was carried out in a separate room equipped with UV light, which is only used for extraction of samples with low DNA concentration. UV light was switched on before and after each isolation procedure, in order to avoid contaminations. DNA was isolated from scats using the QIAamp DNA Stool Mini Kit protocol (QIAGEN®; Appendix I). To monitor for potential DNA contaminations, negative controls (without genetic material, only reagents) were included throughout the entire process.

Polymerase chain reaction (PCR) reagents were combined in a different room apart from the extraction room, using aerosol resistant pipette tips and sterilized material in a DNA UV-cleaner box, to ensure complete sterilization. A fragment corresponding to a non-coding region (D-loop region) of mitochondrial DNA (mtDNA) was amplified for all samples by PCR. This region contains the main regulatory elements for the replication and expression of the mitochondrial genome (Sbisà et al., 1997). Amplification was performed using three different pairs of primers: L-Pro (Mucci et al., 2004) and MelCr6 (Marmi et al., 2006) for all samples; Thr-L 15926 and DL-H 16340 (Vilà et al., 1999) and CR1 and CR2R (Palomares et al., 2002) for samples that were not successfully amplified using the previous primers. Amplifications were performed in a final volume of 25µL using: 1-5µl of DNA template; 2µg/µl of BSA; 2mM $MgCl_2$; and 0,12µM of each primer solution. Thermocycling parameters for the first two pairs of primers consisted of: an initial activation step at 94◦C for 3min; 42 cycles of denaturation at 94◦C for 1min, annealing at 48◦C for 2min and extension at 72◦C for 1min 30s; and a final extension at 72◦C for 10min. Thermocycling parameters for primers CR1 and CR2R consisted of: an initial activation step at 95◦C for 15min; 40 cycles of denaturation at 95◦C for 30s, annealing at 58◦C for 20s and extension at 72◦C for 20s; and a final extension at 60◦C for 10 min, following Monterroso et al. (2013).

PCR success was confirmed through electrophoresis and visualization of DNA fragments under UV light. Successfully amplified fragments were enzymatically purified (ExoSap-IT®). Sequencing was performed using the above-mentioned primers. The resulting sequences were analysed using the software MEGA (Molecular Evolutionary Genetics Analysis) version 7 (Kumar et al., 2016) and identification was performed by comparing generated sequences with sequences deposited in GenBank® using BLAST (NCBI, 2017). For species identification, and before DNA extraction, it was confirmed that

all mesocarnivores had sequences recorded in the GenBank®. The sequenced samples were accepted as being of a mesocarnivore species when the match with the sequences in the database was ≥98% (Hebert et al., 2003; Hubert and Hanner, 2015).

3.3.3. Data analysis

The DNA isolation and amplification success was estimated by the number of successfully amplified samples, relatively to the number of samples from which we tried to isolate DNA. Successfully amplified samples were then sequenced.

Morphological identification of the collected scats was performed in two ways: by four individual researchers and by two teams (association) of two researchers (experienced *vs* not experienced). The precision of this conventional approach was expressed as the proportion of correct identifications ("matches" with molecular identification), over the total number of samples with success in molecular identification. The absence of decision based on morphological criteria were taken into account (as identification error) for the following calculations. For identification based in more than one observer, when the observers were not in agreement, the identification was based on the identification of the most experienced observer.

The identification success rate from different observers (individual and team) were compared using Chi-square proportion tests (Armitage, 1966). The p-values of the multiple tests was adjusted using Bonferroni correction, in order to reduce Type I errors due to multiple testing (Gordon et al., 2007). Morphological identifications based on the data with the highest species identification success rate, was then selected for the estimation the success of identification for each species. The specific success rate was estimated based on the number of morphological identifications that matched the molecular identification, over the total number of samples assigned to the species, based on morphological criteria.

3.4. Results

We collected 96 scats that were initially assigned to seven species: red fox, Iberian wolf (*Canis lupus signatus*), weasel (*Mustela nivalis*), stone marten, European badger (*Meles meles*), common genet (*Genetta genetta*) and wildcat (*Felis silvestris*). DNA isolation was attempted in 63 samples (66% of the collected scats), selected based on their age and conservation. Of the 63 analysed samples, 52 (83%) were successfully amplified, sequenced and assigned to mesocarnivores species based on molecular criteria.

The success of morphological identification among the researchers varied between 48% and 67% (52% to 33% error rates, respectively). Experienced researchers obtained highest success rates (Figure III.1).

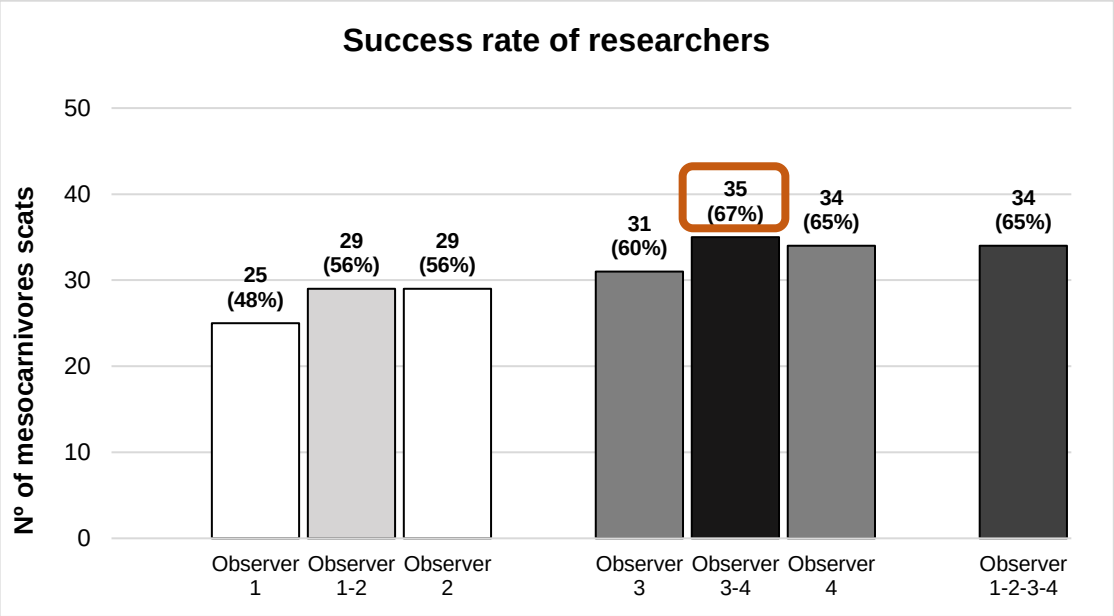

Figure III.1 – Individual and team success rate of researchers, in the morphological identification of mesocarnivores scats. According to the level of expertise, the observers were grouped in teams of different levels of knowledge: non-experienced researchers (observers 1 and 2 – light coloured bars) and experienced researchers (observers 3 and 4 – dark coloured bars). Bar representing more than one observer show the consensus data between observers.

Although there were no significant differences between the success rates among experienced and non-experienced researchers (χ^2=1.016, df=1, p> 0.05), in order to calculate the success of identification in each species, we selected the morphological identification data with the highest species identification success rate, i.e. association of the identifications of experienced researchers, corresponding to 67% (Figure III.1).

3.4.1. Species identification

Agreement between morphological and molecular identification of scats varied among species (Table III.1).

Table III.1 – Matches and mismatches among identifications based on morphological and molecular criteria, for all the 52 samples for which molecular information was available. Percentages of times that: a scat is miss-assigned to a given species – false positive – (species is overrepresented, weighted across row); and a scat of a given species is assigned to another species – false negative – (species is underrepresented, weighted across column), are also presented in the last column and row, respectively.

		Species inferred by genetic analysis						False positive rate
		Vulpes vulpes (*n*=38)	*Martes foina* (*n*=8)	*Martes martes* (*n*=2)	*Meles meles* (*n*=1)	*Genetta genetta* (*n*=1)	*Canis lupus familiaris* (*n*=2)	
Species inferred by morphological criteria	*Vulpes vulpes* (*n*=28)	**27**	-	-	-	-	1	1/28 (4%)
	Canis lupus signatus (*n*=1)	1	-	-	-	-	-	1/1 (100%)
	Mustela nivalis (*n*=3)	-	2	1	-	-	-	3/3 (100%)
	Martes foina (*n*=16)	8	**6**	1	-	-	1	10/16 (63%)
	Meles meles (*n*=1)	-	-	-	**1**	-	-	0/1 (0%)
	Genetta genetta (*n*=1)	-	-	-	-	**1**	-	0/1 (0%)
	Felis silvestres (*n*=2)	2	-	-	-	-	-	2/2 (100%)
False negative rate		11/38 (29%)	2/8 (25%)	2/2 (100%)	0/1 (0%)	0/1 (0%)	2/2 (100%)	

Most of the 52 genetically identified scats belonged to red foxes (*n*=38). The remaining samples were molecularly assigned to stone marten (*n*=8), domestic dog (*Canis lupus familiaris*; *n*=2), pine marten (*n*=2), common genet (*n*=1) and European badger (*n*=1).

Error rates in scats' species assignment based on morphology were highly variable and can happen in two ways: 1) when a scat is miss-assigned to a given species – false positive – (the species is overrepresented); and 2) when a scat of a given species is miss-assigned to another species – false negative – (the species is underrepresented). In the first situation, the error rate varies from 0% for the badger and genet samples, to 100% for the weasel or wildcat (Table III.1, last column). Regarding the second situation – when a scat of a given species was miss-assigned to other species – the rate was also highly

variable, ranging from 0% (common genet and European badger), 25% and 29% (in stone marten and red fox) to 100% (Iberian wolf and pine marten; Table III.1, last row).

Most of the sequenced scats were identified by morphological characteristics as belonging to the red fox (54%, *n*=28) and to the stone marten (31%, *n*=16). The samples that were identified by the observers (morphological identification) as belonging to the red fox had an accuracy rate of over 96% (27 out of 28), and the molecular identification revealed that the only misidentified sample belonged to the domestic dog. However, 29% (11/38) of scats that were identified as belonging to the red fox by molecular analysis, were miss-assigned to another species by field observers – Iberian wolf (*n*=1), stone marten (*n*=8) and wildcat (*n*=2). The samples that were identified as belonging to the stone marten were accurately identified, by morphological characteristics, in 38% (6 out of 16) of the occasions. The molecular analysis revealed that some samples were morphologically misidentified as belonging to the stone marten, when in fact they belonged to the red fox (*n*=8), pine marten (*n*=1) and domestic dog (*n*=1). It was also revealed that, 25% (2/8) of scats that were molecularly identified as stone marten, were miss-assigned to the weasel.

Through molecular identification it was possible to infer that all samples assigned to Iberian wolf, weasel and wildcat, in the field, were misidentified. Based on molecular identification, the Iberian wolf's scat was assigned to the red fox (Figure III.2 – A); the weasel scats were assigned to the stone marten (*n*=2) and pine marten (*n*=1); and the two wildcat scats were actually assigned to the red fox (Figure III.2 – B). The samples belonging to pine marten and domestic dog were always miss-assigned to other species, i.e. they were never identified in the field (see Table III.1).

A **B**

Figure III.2 – Photographic records of scats genetically identified as red fox, but that were mistakenly identified by morphological criteria. **A** – Misidentified was Iberian wolf; **B** – Misidentified was wildcat. Photo credits: Dário Hipólito.

3.5. Discussion

Scats are a very useful tool in ecology, conservation and monitoring of species (Kohn and Wayne, 1997). However, if not accurately identified, they can generate misleading ecological patterns. The results of the current study evidenced that the identification of species, through a non-invasive sampling method, is species specific and can lead to success rates in scats' species assignment.

Taking into account that the different errors that may occur in the identification of scats – when a scat is miss-assigned to a given species and when a scat of a given species is miss-assigned to another species –, in the sampling data, some species may be overrepresented (first error) or underrepresented (second error). Our results show that, in some cases, the success rate in scats' assignment is 100% (common genet and badger), while for other species the rate is lower, being 0% in the case of the Iberian wolf, weasel and wildcat. Nevertheless, it is necessary to be careful in the results inferences, since for some species the number of samples is really low. However, the percentage of scats identification success rate for the two species that we had more samples were highly contrasting – the red fox is underrepresented (4% of the scats that were identified in the field as belonging to the fox, were wrongly assigned to this species; but 29% of the samples that were molecularly identified as belonging to the red fox, were wrongly assigned to other species); the stone marten is overrepresented (63% of the scats that were identified as belonging to the stone marten, by morphological criteria, were misidentified; and 25% from stone marten' scats were miss-assigned to other species) –, indicating that the specificity in success rates can really be a pattern for mesocarnivores.

The variation in success rate may be due to several factors. According to Davison et al. (2002), the smaller the population the greater the error in the scat identification by the observers, which may justify the low success rate detected in the identification of Iberian wolf and weasel. On the other hand, the high abundance of a species may be responsible for its high detection rate, which is the situation of the red fox (Monterroso et al., 2011). Even though, according to our results, the red fox is an underrepresented species, since 29% of its scats were miss-attributed to other species. Depending on their diet, wildcat scats can be confused with scats from other mesocarnivores, especially red foxes (Urra et al., 2014), which is in agreement with the data of this study. Furthermore, different food searching strategies could lead to a variation of the morphological characteristics of scats in the same population (Monterroso et al., 2013), and distinct climatic condition can influence the preservation of scats shape through time, which constrains the accuracy of species identifications. In many cases, the researchers use other environmental clues to

determine visually scat's species. For example, badgers deposit their scats in latrines composed by several pits located on the path borders (Roper, 2010), used only by badger. This behavior will enhance scats identification rate for this mustelid, a pattern evidenced by our data.

Of the 52 genetically identified scats, the success of identification among the observers varied between 48% and 67%. The level of expertise has not turned out to be a factor with statistical significance in the success of morphological identification, as reported by Bulinski and McArthur (2000), but there is some inter-observer variation that should be recognised and that can introduce bias in identification an in posterior data analysis (e.g. Species Distribution Modelling; Molinari-Jobin et al., 2012). Poor identification is reported as a recurrent problem (Dalén et al., 2004; Davison et al., 2002), and this is a concern for researchers because of the uncertainty that it causes in the results. The genetic identification can be an adequate case-solving approach (Beja-Pereira et al., 2009; Harrington et al., 2010) and is strongly advisable, in particular for species for which success rates are very low. The success of DNA isolation, amplification and molecular identification obtained a high success rates (83%), agreeing with previous studies: 72% in Fernandes et al. (2008), 78.4% in Monterroso et al. (2013) and 81.1% in Oliveira et al. (2010). Despite being a rigorous process, it is not 100% effective. The high temperatures that may occur in our study area, during summer season, contribute to the rapid degradation of scat DNA (Santini et al., 2007) and reduce the effectiveness of the process. As the DNA obtained from the non-invasive samples method is generally in low quantity and often contaminated and degraded (Broquet et al., 2007), the optimization of the technique is essential, to achieve greater and faster success (Nakamura et al., 2017).

In conclusion, the variation detected in the success rate of identified scats for the different species, confirms the importance and need to use more accurate methods. Thus, using molecular techniques as a tool in ecological research, can help generate a clearer picture on different aspects of carnivore ecology (e.g. diet, habitat preferences), being complementary to the morphological identification of scats. In a time when wildlife face continuous and multifactor conservation problems, managers cannot afford to base conservation actions on erroneous ecological data.

Chapter IV

This chapter is being prepared to be submitted to ***Ethology, Ecology & Evolution***, as an "Original article".

4. "Humans do matter: determinants of red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) presence in a western Mediterranean landscape"

4.1. Abstract

Habitat loss, due to landscape changes induced by human activities, is considered one of the main drivers of biodiversity decline. Thus, the development of conservation strategies is an essential measure to overcome this problem. Mesocarnivores, as higher trophic level species with crucial roles in ecosystems functioning, are a pivotal functional group in such strategies. However, effective conservation strategies need to be based on a fair understanding of the species ecological patterns and the processes that determined it. Generalist mesocarnivores, such as the red fox (*Vulpes vulpes*) and stone marten (*Martes foina*), are excellent models to study species ecological adaption to landscapes moulded by humans. Using GLMM (Generalized Linear Mixed Model), we aim to understand how the landscape context affects the presence of these generalist species, in a Mediterranean ecosystem (northeastern Portugal) characterized by a combination of natural areas and traditional agricultural land uses. Based on scats' distribution, we generated distribution models to test different ecological hypotheses affecting those species presence – anthropic disturbance factors, altitude and landcover patterns. While anthropic activities have a negative influence on stone martens' presence (disturbance effect), the proximity to urban areas has a positive effect due to an increase of food availability and to avoid competition with ecological similar species. Red foxes seem to have two distinct strategies: in forest dominated areas (>75% of landcover), human activities have a negative effect (disturbance); in areas with a low percentage of forests (<25%), agriculture patches and the proximity to urban areas are the principal factors determining it presence, with a positive influence (food). Our results confirm the opportunistic character of these two species and their high degree of adaptation, demonstrated by the fact that while they both tend to avoid anthropic disturbance, they move toward settlements to take advantage of surplus associated to human food. Further, the results also reveal that, at least for the red fox, landscape context influences the detected pattern. Consequently, considering that populations of the same species are constrained by the same factors, independently of the landscape composition, will affect the efficacy of management measures put in place to assure the regional survival of a species.

Keywords

Altitude – Conservation – Disturbance factors – Landscape heterogeneity – Mesocarnivores

4.2. Introduction

In last decades, the human population has been growing at a fast rate (United Nations, 2014). This phenomenon led to a high expansion, both in size and number, of urban areas and agricultural lands, due to an increasing demand for resources, menacing global biodiversity and making the preservation of ecosystems one of the greatest challenges of the future (Barnosky et al., 2011). Changes that occur in a single habitat can modify the composition and ecological relations of species inhabiting it, creating a chain of events that affects the entire ecosystem (Pereira et al., 2012). Thus, understanding how landscape changes affect species presence, as well as assessing the mechanistic link between environmental and anthropic factors and the distribution of species, are one of the main objectives in ecology (Manel et al., 2001). Disentangling such relation allows determining species resilience to environmental disturbances (ecological adaptation or versatility; MacNally, 1995) and delineate effective conservation plans and sustainable development strategies (Gheler-Costa et al., 2016).

Carnivores species have an important role in ecosystem functioning (Gittleman, 1989), and changes in their guild composition have cascading effects upon all community. Therefore, they are often a target group for the application of landscape monitoring and management programs (Barea-Azcón et al., 2007a), being frequently used in conservation by proxy approaches (Caro, 2010). However, the guild is composed by very distinct species, which may range from highly specialists and carnivorous (such as many felids) to generalist and ubiquitous species (e.g., medium size canids; Nowark, 2005). Consequently, their sensitivity to changing conditions is also very different. While species, such as the wildcat (*Felis silvestris*), often avoid areas subject to anthropic disturbance (Monterroso et al., 2009), others, like the European badger (*Meles meles*), use frequently human structures and food (e.g. game species feeding structures; Hipólito et al., 2016). Landscape composition and change, often induced by human, is also an important factor affecting differently carnivore spatial structure – as species have different ecological requirements (e.g. food or shelter), that are fulfilled by different habitats in distinct seasons (Cruz et al., 2015; Kalle et al., 2014). Furthermore, both factors (i.e. anthropic disturbance and landscape composition) can act synergistically with environmental drivers, such as altitude,

in shaping carnivore presence pattern – as altitude influence temperature and terrain slope, these factors affect landscape's characteristics, human use and disturbance levels (Martin et al., 2010; Silva et al., 2013).

The importance of understanding carnivore ecological responses to altered environments is enhanced in areas where human's activities are moulding the landscape for centuries or millenniums, such as in the Mediterranean Europe (Blondel, 2006). In Mediterranean spatial and temporal heterogeneous landscapes, different habitat mosaics provide different resources availability throughout the year, and species presence can thus be dependent of the landscape composition context (e.g. Silva et al., 2017). In Portugal, the carnivore guild is diverse and composed by 15 species (Álvares et al., 2017), including habitat specialists as the pine marten (*Martes martes*), and generalist as the red fox (*Vulpes vulpes*) or the stone marten (*Martes foina*; Loureiro et al., 2012). Generalist species, which are more resilient and have the ability to use a wide range of resources, are good models to understand how disturbance, land composition and environmental factors influence the distribution of carnivore species, especially in a global change scenario (Sinclair et al., 2010).

Therefore, our objective is to understand what factors might be constraining the presence of two generalist mesocarnivores occurring in sympatry in the Iberian Peninsula – the red fox and the stone marten (Palomo et al., 2007) – and if and how the landscape context influences the distribution patterns. We used a Species Distribution Models (SDM) approach that allow combining field observations of species occurrence with environmental factors variations (Elith and Leathwick, 2009). In order to achieve our goal, we formulated four different ecological hypotheses regarding what might be influencing the presence of red fox and stone marten, in a human-influenced Mediterranean landscape, and the relevance of considering the landscape context: i) Disturbance hypothesis, i.e. the anthropic disturbance factors are the main factors that influencing the presence of species (H1; Curveira-Santos et al., 2017; Santos and Santos-Reis, 2010); ii) Altitude hypothesis, i.e. the species distribution is influenced by altitude factor (H2; Silva et al., 2017; Zabala et al., 2009); iii) Landcover composition hypothesis, i.e. the cover and composition of the land is the most important determinant of the species distributions (H3; Curveira-Santos et al., 2017; Pereira et al., 2012); and iv) Hybrid hypothesis, i.e. when a combination of factors associated with the previous hypothesis, validated the detected pattern (H4).

4.3. Material and methods

4.3.1. Study area

The study was conducted in Bragança and Vila Real districts (41º30'33.3"N, 6º56'57.5"W), covering a total area of 8,395 Km^2 (Figure II.1) and located on the northeastern corner of Portugal (Fleskens et al., 2009)**.** The climate, according to Köppen-Geiger's classification, is warm temperate with dry and warm summers (Csb; Kottek et al., 2006) and the region's orography (i.e. an interior plateau, limited by high mountains on it north and west border and River Douro on the south and east ends) has an influence on the climate by creating a condensed and humid environment (Everpidou et al., 2010).

The land cover is highly diversified, being possible to find deciduous forests (*Castanea sativa*, *Quercus pyrenaica*, *Quercus rotundifolia*, *Quercus suber*)**,** conifer forests (*Juniperus oxycedrus, Pinus pinaster, Pinus sylvestris, Pseudotsuga menziesii*), scrublands (*Cistus ladanifer, Cytisus spp., Erica tetralix*, *Genista anglica)* and permanent natural meadows (*Festuca indigesta*, *Oxalis pes-caprae*; Azevedo, 2012; ICNF, 2017a). Furthermore, the landscape is also characterized by extensive and traditional agricultural activities and by livestock grazing systems (Everpidou et al., 2010; Oliveira Baptista et al., 2003).

The study area is one the Portuguese regions encompassing a highly diverse carnivores community (Álvares et al., 2017). This is one of the few areas, at national level, where the presence of Iberian wolf (*Canis lupus signatus*, classified as "Endangered") has been confirmed, as well as of the ermine (*Mustela erminea*) and pine marten, both classified as "Data Deficient" by the Portuguese Red List Book (Cabral et al., 2005; Matos, 2012; Sales-Luís, 2012). Moreover, this Northeastern region of Portugal also hosts sympatric populations of wild boars (*Sus scrofa*), roe deers (*Capreolus capreolus*) and deers (*Cervus elaphus*), which coexist with domestic herbivores (e.g., sheep, goat, cattle), raised mostly in an extensive regime (Everpidou et al., 2010).

The region encompasses several protected areas, such as the Montesinho Natural Park and the International Douro Natural Park (Figure II.1), which are part the National Network of Protected Areas (ICNF, 2017c).

4.3.2. Fieldwork and characterization of sampling points

The study area was divided into a grid of 10x10km and, using a knight chess moving pattern, starting on the northeast corner of the area, we selected 15 squares of 10x10km. Each one of these squares was once gain divided into 4 grid cells of 5x5km. Only two of 5x5km grid cells per 10x10km grid square, were randomly selected to be sampled.

Sampling was performed in summer (July–September 2016), when the majority of mesocarnivores' offspring is becoming independent (Loureiro et al., 2012), and their traces are easier to find. During this period, we monitored a total of 143 line transects, each 500m long, randomly distributed throughout the 5x5km grid cells (4 or 5 transects per grid cell) to adequately sample all existing habitats (Figure II.1). Transects were located along trails or dirt roads, surveyed on foot by one or two observers to search for mesocarnivores' signs of presence (scats and footprints).

For each scats, footprints or direct observation of the animals, we recorded: the ID of the grid square and the transect; date; weather conditions; a description of the vegetation surrounding the detected sign (within a radius of 10m); the anthropic threats found in it vicinity (e.g. construction debris, chemical wastes); the conservation status of the samples (degree of freshness – only for scats); geographical coordinates and altitude of each sign; photographic record code; and sample code (Appendix II – Table VII.1). All the evidences were initially identified in the field based on their location, morphology, odour and dimensions with the support of field guides (Bang and Dahlstrom, 2006; Sanz, 2003), and later by other observers, through the analysis of photographic records.

A total of 291 mesocarnivores evidences (279 scats, 10 footprints and 2 sightings) were recorded. To minimize scats' identification errors, we applied an ensemble solution. First, we estimated the success rate of scats identification based on the morphological criteria for all individual observers and their combined decisions, using as reference the molecular identification of scats (see previous chapter). We then use the data for which the highest species identification success rate was achieved – i.e. identifications determined by the combined opinions of both experienced researchers.

All red fox or stone marten signs of presence were included in a Geographical Information System (GIS), using a free and open source Geographic Information System software (QGIS 2.18.1 – Las Palmas version; Quantum GIS, 2016). To test our working hypotheses, we generated random pseudo-absence points. The pseudo-absence points were generated along the monitored transects, and based on the assumptions that they could not be defined in transects where the presence of the target species had been recorded. Thus, in each transect were no sign of red foxes/ stone martens were detected, three points were defined, at 0m, 250m and 500m. As the number of identified signs and of transects where the species was not detected, varied between both species (see results), the proportion of pseudo-absences also differed (as we were limited by the total number of transects monitored). They represent, approximately, 40% (for the red fox) and 60% (for the

stone marten) of the total sample (i.e. 40% or 60% of pseudo-absence points and 60% or 40% of presence points, for the red fox and stone marten, respectively).

In the case of the red fox, given the error rates detected when comparing morphological and molecular identification, it seems that all signs of presence that were morphologically identified as red foxes, and which have not been molecularly confirmed, are correctly identified, since the percentage of scats, that was false positive, was very low (see previous chapter, Table III.1 last column). In relation to scats that belonged to red foxes, but which were misidentified as belonging to another species (false negative; see previous chapter, Table III.1 last line), as these scats were in the same grid cells of 5x5km (or even on the same transects) as well as other signs of presence of the red fox, the variables that may influence the presence of this species in that place, will already be considered in the models – exclusion of systematic error. Regarding the stone marten, due to the error rates detected in the morphological identification of scats (false positives and false negatives; previous chapter, Table III.1), it is necessary to be careful when drawing the conclusions from the obtained distribution models.

For all points (presence and pseudo-absence) a buffer was defined and centred on the red fox's and stone marten's signs of presence. Buffer size roughly corresponded to the radius of a hypothetical circular core area (most used area within the home range; Pandolfi et al., 1997; Powell, 2000) of the target species in Mediterranean environments, estimated by other authors elsewhere – for the red fox a buffer with an area of 0.11km^2 (Pandolfi et al., 1997), and of 0.5km^2 for stone marten (Santos-Reis et al., 2005). For each buffer, using the GIS and the available land cover information (Land Use and Occupancy Map of Continental Portugal for 2007 - COS2007; DGT, 2010) we estimated the proportion of each land cover category, as well as the distance from its border to the nearest urban area. Five habitat types, based on the categories defined in the COS2007, were considered: artificialized territory (i.e. settlements, houses and industrial facilities); agricultural and agroforestry areas; forests; shrub and herbaceous vegetation patches; bare soil areas or with sparse vegetation; and water bodies and lines.

4.3.3. Data analysis

To test our working hypotheses, we grouped the variables collected during the field work and those extracted from the GIS, according to our perception of their influence on each species presence pattern and the patterns described by other authors (e.g. Pereira et al., 2012; Santos and Santos-Reis, 2010; Zabala et al., 2009). Thus, they were divided the variables into three categories, which correspond to three ecological a priori defined

hypotheses: Human disturbance (Disturbance hypothesis – H1); Altitude (Altitude hypothesis – H2); Landcover (Landcover composition hypothesis – H3; Table IV.1). All presence and pseudo-absence were characterized using each variable described in Table IV.1.

All the continuous variables were standardized, i.e., were transformed to standard scores (*z-scores*), which are a useful way of putting data from different sources onto the same scale, allowing comparisons (Mackenzie et al., 2006).

Table IV.1 – Variables used to test the ecological hypotheses considered as potential explanations for the detected distribution patterns of red foxes and stone martens in Trás-os-Montes region.

	Variable acronym	**Variable description**	**Units**
Disturbance hypothesis (H1)	Art_area	Artificialized area – industrial faciliteis, urban areas, etc.	Percentage (%)
	Debris	Construction debris	Presence (0/1)
	Pollution	Chemistry and physics (trash) pollution.	Presence (0/1)
	Fire	Evidences of recent fire events.	Presence (0/1)
	Infra	Infrastructures – dams, windmills, etc.	Presence (0/1)
	Livestock	Sheep, goat and cattle.	Presence (0/1)
	Machinery	Agriculture machinery – tractors, etc.	Presence (0/1)
	Dist_urban	Distance to the nearest urban area.	Meters (m)
	Vehicles	ATV, motorbikes, etc.	Presence (0/1)
Altitude hypothesis (H2)	Alt	Altitude	Meters (m)
Landcover composition hypothesis (H3)	Agr_area	Agricultural and agroforestry areas	Percentage (%)
	Spars_veg	Areas without or with sparse vegetation	Percentage (%)
	Water	Bayou, lake, puddle, river, etc.	Percentage (%)
	Forest	Deciduous, coniferous and mixed forests	Percentage (%)
	Shrub	Shrub and herbaceous vegetation	Percentage (%)

In spatial modelling, one of the most important data biases is the spatial autocorrelation (Dormann et al., 2007). Thus, we used the Moran I index on the location of each point (presence and pseudo-absence) to test it possible influence on the dependent variable (presence/ pseudo-absence of each species; Carl and Kühn, 2007). Multicollinearity is also another source of bias in SDM (Blalock, 1963). Therefore, the collinearity between the independent variables was analysed through the Spearman's rank correlation coefficient (Zuur et al., 2009). When a high correlation between two variables was detected ($r_s \geq 0.7$), the variable which was less correlated with the dependent variable was excluded from the analysis (Filipe et al., 2002).

To test what variables, or group of variables, could be conditioning the distribution of red foxes and stone martens in the study area, four series of Generalized Linear Mixed Models (GLMM) were created (Zuur et al., 2009), based on the four categories defined above. We used a two-phase analysis. First, and for each of the three first hypotheses (H1-H3), we produced a series of models corresponding to all possible combinations of the candidate variables defined for each group. The model selection process for each hypothesis was based on Akaike's Information Criterion, corrected value for small sample sizes – AICc (Burnham and Anderson, 2002). For each model, the AICc value was calculated and the best models were considered those that presented lower values of AICc (Burnham and Anderson, 2002). All models whose ΔAICc <2 (ΔAICc corresponds to the difference between the AICc of the model and the lowest AICc value obtained) were considered equally best models and, therefore, selected as the best models for each hypothesis (Burnham and Anderson, 2002). When several models met the ΔAICc <2 criterion, the average model was calculated. In a second phase, we selected the variables included in the best models of each hypothesis, whose 95% confidence interval (CI) did not include the zero, to be tested in the hybrid hypothesis. For this hypothesis, it was used the same analytical procedure described above. Finally, we compared the ΔAICc of each of the best models of the four hypotheses, selecting those with the lower AICc as the one(s) that best explains the variability of our dataset. Following Arnold (2010) suggestion, we estimated the relative importance of each variable (sum of the Akaike weights of all models that include the variable) included in the overall best models to exclude uninformative parameters from the discussion.

Specifically for the red fox's data, we re-analysed the data using a different approach because: the combined interpretation of some variables, in the first analysis, seemed contradictory (the proximity to the nearest urban area and the percentage of forest had both a positive influence; see results and Table IV.3); and the continuous variable included in

the best models that assumed a higher coefficient – "Forest" – showed a highly bimodal distribution, with a significant intergroup difference (see results; Figure IV.2). Therefore, we sub-sampled our red fox data according to the percentage of forest within the buffer and defined two-groups: 1) including the presence data with less 25% of forest within the buffer area; and 2) the data for which the percentage of forest was greater than 75%. This division allowed to assess the influence of variables in two distinct and extreme landscapes contexts – areas dominated by forests (more natural/ closed environment) and areas with low percentage of forest (more anthropic/ open environments), dominated by agricultural and artificialized areas. With this approach, we could test the influence of variables whose effect might be masked by the "forest" variable, which may be determining the species presence in different landscape contexts. Thus, for the low forest context, our dataset was composed by 77 presence points, and 51 randomly chosen pseudo-absence points, to assure that the proportion between both point types continued to be 40% of pseudo-absences and 60% of presences. For the high forest context, the number of points, where the presence of red fox was registered, was 57 and the pseudo-absence points 38.

Finally, best model ability to discriminate the data was assessed by producing the model's Receiver Operating Characteristics (ROC) curve, and estimating the Area Under the Curve (or AUC; van Erkel and Pattynama, 1998). High performance models are indicated by large areas under the ROC curve. Usually AUC values of 0.5-0.7 are indicative of low accuracy, values of 0.7-0.9 indicate adequate applications and values of >0.9 indicate high accuracy (Manel et al., 2001).

The statistical analyses were computed using the software RStudio© version 1.0.143 and R version 3.4.0 (R Core Team, 2017), using the extensions "ape" (Paradis et al., 2004), "lme4" (Bates et al., 2015), "MuMIn" (Barton, 2016) and "pROC" (Robin et al., 2011).

4.4. Results

Of the 291 evidences of mesocarnivores recorded during the fieldwork, a total of 212 records (73%) were identified as belonging to red fox and 55 (19%) to stone marten, whose distribution, in the study area, is represented in Figure IV.1.

The red fox has a generalised distribution throughout the study area, as it signs of presence were found in all the largest sampling squares (10x10 km). The stone marten is also present in almost the entire area (with the exception of two 10x10km cells). Evidences of these two species were found in the same grid square and, in some cases, in the same

transect. In the sites where the species were not detected (pseudo-absence), 132 points were generated for the red fox and 83 points for the stone marten, corresponding to 38% and 60% of the total data used in the SDM of each species.

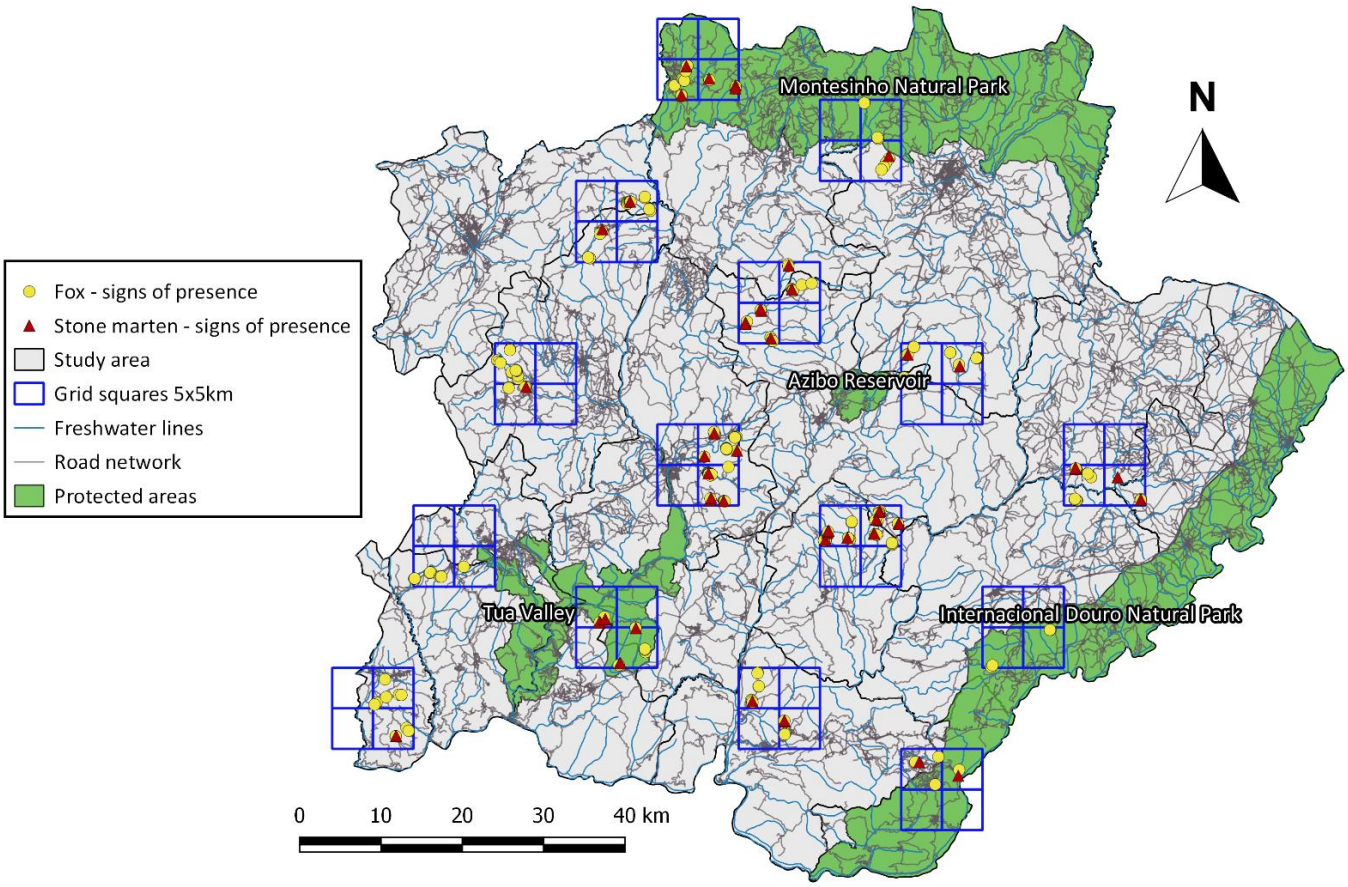

Figure IV.1 – Location of the detected red fox and stone marten signs of presence, inside the sampling grids, along the region of Trás-os-Montes. Protected areas (Natural parks – Montesinho and Internacional Douro; Regional Natural Park – Tua Valley; and Protect landscape – Azibo Reservoir) are also showed.

4.4.1. Distribution models

A significant spatial autocorrelation (Moran I=0.47 for red fox, and Moran I= 0.21 for stone marten; all $p<0.05$) was detected for the presence sites of both species. To overcome the possible bias associated with spatial autocorrelation, data was analysed using GLMM approach (Dormann et al., 2007), using "municipality" (which relates to the municipality where each sign of presence was found within the study area) as a random factor to incorporate the data spatial structure into the models. The analysis of the collinearity between the independent variables of each hypothesis, revealed the absence of significant correlations for those used in the red fox analysis. However, for the stone marten data, a

significant high correlation was detected between “Art_area” and “Dist_urban” variables, used to test the Disturbance hypothesis (H3; Table IV.1). Since the latter variable had a higher correlation with the dependent variable (presence of the species; ρ= -0.050; p=0.56), the variable “Art_area” (ρ=0.013; p=0.88) was excluded from the stone marten modelling procedure.

Red fox (*Vulpes vulpes*)

In an initial phase, we produced a total of 545 models to describe red fox distribution in the study area: 512 associated with the Disturbance hypothesis, one with the Altitude and 32 with the Landcover composition hypothesis (Appendix III). From those, we identified as best model of each hypothesis four, one and two, respectively (Table IV.2).

From all the variables included in the best models for each hypothesis, only eight – the percentage of agriculture, forests and shrubs patches in the buffer area, altitude, distance to the nearest urban area, and the disturbance associated with the presence of fire, cattle and vehicles – showed a coefficient 95% confidence interval (CI) that did not include the zero. Thus, in a second phase, these variables were used to produced models associated with the hybrid hypothesis. From 256 generated hybrid models (Appendix III), two were considered the best models and achieved the lowest AICc of all models produced, with a minimum ΔAICc for the other hypothesis best models of 17.1 (Table IV.2). Thus, the best models that described the presence of the red fox were the ones that were associated to the Hybrid hypothesis, corresponding to the combination of the eight variables, mentioned above, included in the best models of the first three hypothesis (i.e., human disturbance, altitude and landcover composition). The best average model revealed a high adequacy to the data (AUC= 0.91).

Table IV.2 – Best models, for each hypothesis tested, and null model for the distribution of red fox. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. Within each hypothesis, the models are ranked by the ΔAICc value – variation between the AICc of each model and the lower AICc detected for models in the same hypothesis. The ΔAICc Total express the variation between the AICc, of each model, and the lower AICc value among all models of all hypotheses. Finally, the probability of each model being the best in each hypothesis is presented through the Akaike weight. LogLik represents the models' log-likelihood.

Hypothesis	Model	df	LogLik	AICc	ΔAICc	Weight	ΔAICc Total
	Null model	-	-192.9	389.7	-	-	74.2
Disturbance (H1)	Pollution + Livestock + Fire + Machinery + Vehicles + Dist_urban	8	-158.1	332.6	0.00	0.188	17.1
	Pollution + Livestock + Fire + Infra + Machinery + Vehicles + Dist_urban	9	-157.2	332.9	0.28	0.163	17.4
	Pollution + Livestock + Fire + Vehicles + Dist_urban	7	-159.4	333.0	0.47	0.149	17.5
	Pollution + Livestock + Fire + Infra + Vehicles + Dist_urban	8	-158.3	333.1	0.51	0.145	17.6
Altitude (H2)	Alt	3	-190.2	386.4	-	0.845	70.9
Landcover (H3)	Water + Agr_area + Floret + Shrub + Spars_veg	7	-166.8	347.9	0.00	0.512	32.4
	Agr_area + Floret + Shrub + Spars_veg	6	-168.5	349.2	0.33	0.263	33.7
Hybrid (H4)	Agr_area + Alt + Livestock + Vehicles + Dist_urban + Floret + Shrub	9	-148.5	315.5	0.00	0.584	0.0
	Agr_area + Alt + Livestock + Fire + Vehicles + Dist_urban + Floret + Shrub	10	-148.3	317.2	1.63	0.258	1.7

Of all the variables include in the best models of the more supported hypothesis, the fire associated variable is the only whose CI cross zero. Therefore, we cannot conclude whether there is a positive or negative influence of fire on the probability of the red fox being present in a location (Table IV.3). All other variables show a well-defined influence: while the altitude, percentage of agriculture, forest and shrubs have a positive effect on the presence of red fox, the distance to the nearest urban area and the disturbance associated with the presence of cattle and vehicles, have the opposite effect.

Table IV.3 – Variables included in the best models of the more supported hypothesis (Hybrid hypothesis – H4), produced to explain the distribution pattern of the red fox (*Vulpes vulpes*), and it coefficients values, standard error (SE) and 95% confidence interval (CI 95%), as well as the z-value and corresponding p-values (*p*), and relative importance. Variables whose CI 95% did not include the zero are represented in **bold**.

Variable	Coefficient	SE	z-value	*p*	CI 95%	Relative importance
(Intercept)	1.276	0.566	2.247	0.025	0.163/2.390	-
Agr_area	**1.461**	**0.309**	**4.727**	**<0.001**	**0.855/2.066**	**1**
Forest	**1.953**	**0.416**	**4.691**	**<0.001**	**1.137/2.770**	**1**
Shrub	**1.549**	**0.389**	**3.987**	**<0.001**	**0.788/2.311**	**1**
Alt	**0.595**	**0.219**	**2.716**	**0.007**	**0.166/1.024**	**0.94**
Dist_urban	**-0.654**	**0.232**	**2.813**	**0.005**	**-1.109/-0.198**	**0.98**
Fire	-0.641	0.920	0.697	0.486	-2.444/1.161	0.32
Livestock	**-1.971**	**0.400**	**4.926**	**<0.001**	**-2.755/-1.187**	**1**
Vehicles	**-3.242**	**1.319**	**2.458**	**0.014**	**-5.827/-0.657**	**0.96**

As mentioned in the methods section, we did a re-analysis of the data using a sub-sampling of our initial data, based on the bimodal distribution of the continuous variable "Forest" (Figure IV.2). After dividing the data according to the percentage of forest within the buffer area (core area), the two extreme groups – locations with less 25% of forest in the buffer area and those where the percentage of forest was greater than 75% – were analysed separately.

The results of the best models and best hypotheses obtained, for these two sub-sampled datasets, are shown in Table IV.4 (forest was <25% of landcover) and Table IV.6 (forest was >75% of landcover).

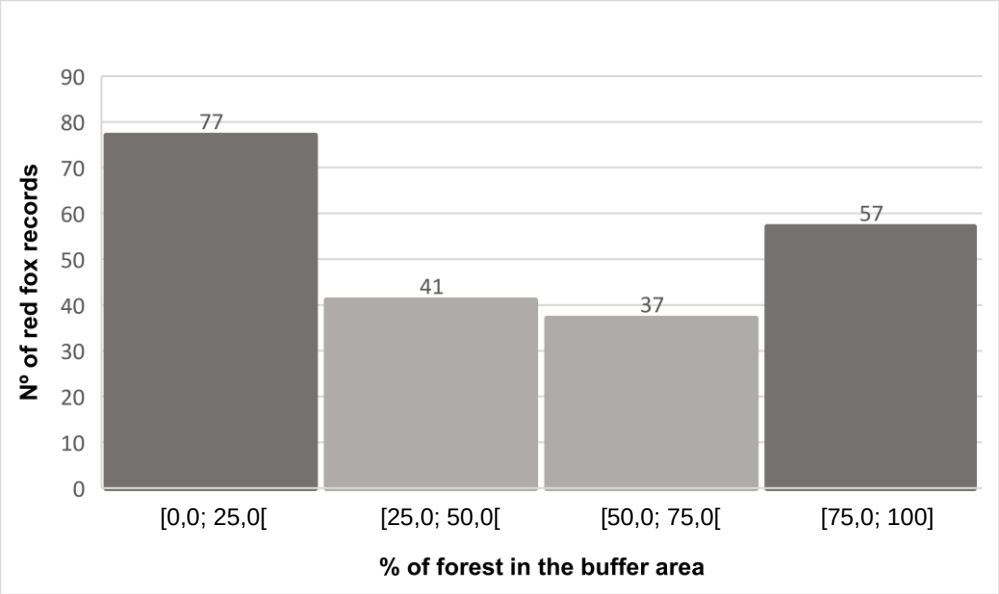

Figure IV.2 – Frequency distribution of the red fox presence data, according to the percentage of forest within the buffer area. Environments with a low percentage of forests (<25%) and forest dominated areas (>75% of landcover), showed a higher frequency of red fox presence – dark gray bars.

When the percentage of forest was <25%, we produced a total of 529 models to describe red fox distribution: 512 associated with the Disturbance hypothesis, one with the Altitude and 16 with the Landcover composition hypothesis (Appendix IV). From those, we identified as best model of each hypothesis ten, one and four models, respectively (Table IV.4). From all the variables included in the best models for each hypothesis, the percentage of agriculture and shrubs patches in the buffer area, distance to the nearest urban area and the disturbance associated with the presence of fire, showed a coefficient 95% CI that did not include the zero. Thus, in a second phase, these variables were used to produced models associated with the Hybrid hypothesis. From the 16 generated hybrid models (Appendix IV), one was considered the best model which had the lowest AICc of all models produced (Table IV.4), with a minimum ΔAICc of 9.8 for the best models of other hypotheses. Therefore, the best model that described the presence of the red fox, in areas with a low percentage of forest, was the one that was associated to the Hybrid hypothesis, revelling a high adequacy to the data (AUC= 0.94).

Table IV.4 – Best models, for each hypothesis tested, and null model for the distribution of red fox, when the percentage of forest in buffer area is less than 25%. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. Within each hypothesis, the models are ranked by the ΔAICc value – variation between the AICc, of each model, and the lower AICc detected for models in the same hypothesis. The ΔAICc Total express the variation between the AICc, of each model, and the lower AICc value among all models of all hypotheses. Finally, the probability of each model being the best in each hypothesis is presented through the Akaike weight. LogLik represents the models' log-likelihood.

Hypothesis	Model	df	LogLik	AICc	ΔAICc	Weight	ΔAICc Total
	Null model	-	-74.9	153.9	-	-	32.2
Disturbance (H1)	Fire + Dist_urban	4	-62.45	133.2	0.00	0.179	11.5
	Livestock + Fire + Dist_urban	5	-61.72	133.9	0.70	0.126	12.2
	Fire + Vehicles + Dist_urban	5	-61.81	134.1	0.89	0.115	12.4
	Pollution + Fire + Dist_urban	5	-61.84	134.3	1.04	0.107	12.6
	Pollution + Livestock + Fire + Dist_urban	6	-60.87	134.4	1.21	0.098	12.7
	Livestock + Fire + Vehicles + Dist_urban	6	-60.93	134.6	1.34	0.091	12.9
	Pollution + Livestock + Fire + Vehicles + Dist_urban	7	-60.02	135.0	1.77	0.074	13.3
	Debris + Fire + Dist_urban	5	-62.25	135.0	1.77	0.074	13.3
	Pollution + Fire + Vehicles + Dist_urban	6	-61.23	135.2	1.93	0.068	13.5
	Fire + Machinery + Dist_urban	3	-62.33	135.2	1.93	0.068	13.5
Altitude (H2)	Alt	3	-74.85	155.9	-	0.274	34.2
Landcover (H3)	Water + Agr_area + Shrub	5	-60.51	131.5	0.00	0.270	9.8
	Water + Agr_area + Shrub + Spars_veg	6	-59.58	131.9	0.35	0.226	10.2
	Agr_area + Shrub	4	-62.09	132.5	1.00	0.164	10.8
	Agr_area + Shrub + Spars_veg	5	-61.32	133.1	1.63	0.119	11.4
Hybrid (H4)	Agr_area + Fire + Dist_urban + Shrub	6	-54.52	121.7	0.00	0.763	0.0

In this case, as in the general distribution of the red fox, the best model to explain the distribution of this species, in environments where the forest percentage was very low, was the Hybrid hypothesis. All the variables that integrate the best models of this hypothesis show a CI 95% that did not include the zero, and therefore we can clearly determine their effect on the dependent variable (Table IV.5). The percentage of agriculture was shown to be the only variable with a positive influence, while the presence of shrub, the threat of fire and distance to the urban areas have a negative effect (Table IV.5).

Table IV.5 – Variables included in the best models of the more supported hypothesis (Hybrid hypothesis – H4), produced to explain the distribution pattern of the red fox (*Vulpes vulpes*) when the percentage of forest in buffer area is less than 25%, and it coefficients values, standard error (SE) and 95% confidence interval (CI 95%), as well as the z-value and corresponding p-values (*p*), and relative importance.

Variable	Coefficient	SE	z-value	*p*	CI 95%	Relative importance
(Intercept)	0.448	0.651	0.688	0.491	-0.828/1.724	-
Agr_area	**1.003**	**0.411**	**2.440**	**0.015**	**0.197/1.808**	**0.95**
Shrub	**-0.946**	**0.339**	**-2.793**	**0.005**	**-1.610/-0.282**	**0.97**
Dist_urban	**-1.047**	**0.433**	**-2.418**	**0.016**	**-1.895/-0.198**	**0.91**
Fire	**-3.089**	**1.293**	**-2.388**	**0.017**	**-5.624/-0.554**	**0.91**

When the percentage of forest was >75%, a total of 521 models describing red fox distribution were generated: 512 associated with the Disturbance hypothesis, one with the Altitude and eight with the Landcover composition hypothesis (Appendix V). For each hypothesis tree, one and two were considered best models, respectively (Table IV.6). From all the variables included in the best models for each hypothesis, six variables – percentage of agriculture and shrubs in the buffer area, altitude, distance to the nearest urban area, and the disturbance associated with the presence of fire, cattle and machinery – presented a coefficient 95% CI that did not include the zero. These variables were used to produce a total of 64 models associated with the Hybrid hypothesis, of which six were considered the best models (Table IV.6). The minimum AICc difference to the best models of the other hypotheses was 5.6 (Table IV.6). Thus, the best hypothesis that described the presence of the red fox in areas with a high percentage of forest was the Hybrid hypothesis. The best average model revealed a high adequacy to the data (AUC= 0.99).

Table IV.6 – Best models, for each hypothesis tested, and null model for the distribution of red fox, when the percentage of forest in buffer area is greater than 75%. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. Within each hypothesis, the models are ranked by the ΔAICc value – variation between the AICc, of each model, and the lower AICc detected for models in the same hypothesis. The ΔAICc Total express the variation between the AICc, of each model, and the lower AICc value among all models of all hypotheses. Finally, the probability of each model being the best in each hypothesis is presented through the Akaike weight. LogLik represents the models' log-likelihood.

Hypothesis	Model	df	LogLik	AICc	ΔAICc	Weight	ΔAICc Total
	Null model	-	-50.6	105.1	-	-	29.1
Disturbance (H1)	Livestock + Fire + Machinery + Vehicles	6	-36.86	86.7	0.00	0.227	10.7
	Pollution + Livestock + Fire + Machinery + Vehicles	7	-36.45	88.2	1.54	0.105	12.2
	Livestock + Fire + Machinery	5	-38.98	88.7	1.97	0.085	12.7
Altitude (H2)	Alt	3	-49.19	104.6	-	0.578	28.6
Landcover (H3)	Agr_area + Shrub	4	-36.57	81.6	0.00	0.533	5.6
	Agr_area + Shrub + Spars_veg	5	-36.39	83.5	1.88	0.208	7.5
Hybrid (H4)	Agr_area + Livestock + Fire + Shrub	6	-31.50	76.0	0.0	0.217	0.0
	Agr_area + Livestock + Fire + Machinery + Shrub	7	-30.80	76.9	0.93	0.137	0.9
	Agr_area + Alt + Livestock + Fire + Shrub	7	-30.92	77.2	1.18	0.121	1.2
	Agr_area + Alt + Livestock + Fire + Machinery + Shrub	8	-29.81	77.3	1.35	0.111	1.3
	Agr_area + Livestock + Fire	5	-33.34	77.4	1.39	0.108	1.4
	Agr_area + Livestock + Fire + Machinery	6	-32.43	77.8	1.86	0.086	1.8

As before, the best model to explain the distribution of this species, in environments where the forest percentage was very high, was the Hybrid hypothesis, being formed by variables from human disturbance, altitude and land use patterns categories. In cases

where the forest percentage was >75%, of all candidate variables, the percentage of agriculture is the only whose CI doesn't cross zero, having a negative influence on the red fox's presence (Table IV.7). For all the other variables, we cannot conclude whether they have a positive or negative influence. However, the variables related to the disturbance appear to have a negative effect on distribution of red fox, as the CI tend to include mostly negative values (Table IV.7).

Table IV.7 – Variables included in the best models of the more supported hypothesis (Hybrid hypothesis – H4), produced to explain the distribution pattern of the red fox (*Vulpes vulpes*) when the percentage of forest in buffer area is greater than 75%, and it coefficients values, standard error (SE) and 95% confidence interval (CI 95%), as well as the z-value and corresponding p-values (*p*), and relative importance. Variables whose CI 95% did not include the zero are represented in **bold**.

Variable	Coefficient	SE	z-value	*p*	CI 95%	Relative importance
(Intercept)	1.958	1.684	1.147	0.252	-1.388/5.305	-
Agr_area	**-2.152**	**0.831**	**2.555**	**0.011**	**-3.802/-0.501**	**1**
Shrub	1.195	0.769	1.533	0.125	-0.333/2.723	0.72
Alt	0.633	0.535	1.165	0.244	-0.431/1.697	0.46
Fire	-5.014	2.570	1.924	0.054	-10.123/0.095	0.87
Livestock	-3.957	2.753	1.421	0.155	-9.416/1.502	0.83
Machinery	1.156	3.192	0.975	0.330	-3.189/9.500	0.47

Stone marten (*Martes foina*)

In an initial phase, we produced a total of 289 models to describe stone marten distribution in the study area: 256 associated with the Disturbance hypothesis, one with the Altitude and 16 with the Landcover composition hypothesis (Appendix VI). From those, we identified as best models of each hypothesis eight, one and seven models, respectively (Table IV.8).

From all the variables included in the best models for each hypothesis, only four – the percentage of agriculture and shrubs patches in the buffer area, distance to the nearest urban area and the disturbance associated with the presence of livestock – showed a coefficient 95% confidence interval (CI) that did not include the zero. Thus, in a second phase, these variables were used to produced models associated with the Hybrid hypothesis. From 16 generated hybrid models (Appendix VI), four were considered the best models for this hypothesis (Table IV.8).

The best models that described the presence of the stone marten in the study area were associated to the Disturbance hypothesis (i.e., showed the lowest AICc of all models produced), with a minimum ΔAICc Total of 5.0 for the best models of the other tested hypotheses (Table IV.8). The validation of the best average model, through the ROC curve, revealed that this was an adequate model (AUC=0.87).

Table IV.8 – Best models, for each hypothesis tested, and null model for the distribution of stone marten. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. Within each hypothesis, the models are ranked by the ΔAICc value – variation between the AICc, of each model, and the lower AICc detected for models in the same hypothesis. The ΔAICc Total express the variation between the AICc, of each model, and the lower AICc value among all models of all hypotheses. Finally, the probability of each model being the best in each hypothesis is presented through the Akaike weight. LogLik represents the models' log-likelihood.

Hypothesis	Model	df	LogLik	AICc	ΔAICc	Weight	ΔAICc Total
	Null model	-	-85.9	175.9	-	-	21.5
Disturbance (H1)	Livestock + Fire + Vehicles + Dist_urban	6	-70.88	154.4	0.00	0.174	0.0
	Livestock + Fire + Vehicles	5	-72.24	154.9	0.53	0.133	0.5
	Livestock + Fire + Dist_urban	5	-72.25	155.0	0.55	0.132	0.6
	Pollution + Livestock + Fire + Vehicles + Dist_urban	7	-70.21	155.3	0.87	0.113	0.9
	Pollution + Livestock + Fire + Vehicles	6	-71.54	155.7	1.31	0.090	1.3
	Livestock + Fire + Infra + Vehicles + Dist_urban	7	-70.44	155.7	1.33	0.089	1.3
	Livestock + Fire	4	-73.82	155.9	1.53	0.081	1.5
	Livestock + Fire + Infra + Dist_urban	6	-71.84	156.3	1.91	0.067	1.9
Altitude (H2)	Alt	3	-85.95	178.1	-	0.260	23.7
Landcover (H3)	Water + Spars_veg	4	-82.63	173.6	0.00	0.182	19.2
	Water + Agr_area + Spars_veg	5	-81.79	174.0	0.46	0.145	19.6
	Water	3	-83.93	174.0	0.48	0.144	19.6
	Water + Agr_area	4	-83.11	174.5	0.95	0.113	20.1
	Agr_area + Spars_veg	4	-83.40	175.1	1.54	0.085	20.7
	Spars_veg	3	-84.61	175.4	1.83	0.073	30.0
	Agr_area + Shrub + Spars_veg	5	-82.53	175.5	1.94	0.069	30.1

Hybrid (H4)	Agr_area + Livestock + Dist_urban	5	-74.49	159.4	0.0	0.321	5.0
	Livestock + Dist_urban	4	-75.60	159.5	0.06	0.311	5.1
	Agr_area + Livestock + Dist_urban + Shrub	6	-74.27	161.2	1.73	0.135	6.8
	Livestock + Dist_urban + Shrub	5	-75.46	161.4	1.94	0.122	7.0

All the variables that have the most influence in the presence of this species are the those related to the human disturbance – the distance to the nearest urban area and the disturbance associated with the presence of pollution, livestock, fire, infrastructures and vehicles (Table IV.9). However, the only variable that has a clear negative influence (CI 95% includes only negative values), is the presence of livestock. The distance to the urban area also seems to have a tendency for a negative effect in the species presence, as despite the confidence interval including the zero, most of the interval is negative.

Table IV.9 – Variables included in the best models of the more supported hypothesis (Disturbance hypothesis – H1), produced to explain the distribution pattern of the stone marten (*Martes foina*), and it coefficients values, standard error (SE) and 95% confidence interval (CI 95%), as well as the z-value and corresponding p-values (*p*), and relative importance. Variables whose CI 95% did not include the zero are represented in **bold**.

Variable	Coefficient	SE	z-value	*p*	CI 95%	Relative importance
(Intercept)	0.332	0.360	0.913	0.361	-0.381/1.045	-
Pollution	1.503	1.236	1.205	0.228	-0.941/3.947	0.35
Dist_urban	-0.516	0.288	1.774	0.076	-1.086/0.054	0.60
Fire	-17.75	2.35E5	0.000	0.999	<-0.001/<0.001	0.92
Infra	-0.715	0.758	0.934	0.350	-2.214/0.784	0.31
Livestock	**-2.010**	**0.508**	**3.922**	**<0.001**	**-3.015/-1.006**	**0.95**
Vehicles	-16.62	2.76E3	0.006	0.995	<-0.001/<0.001	0.25

4.5. Discussion

Mesocarnivores communities have a complex structure, and the species-environment interaction patterns show a spatio-temporal variation due to exposure to new and changing conditions (Díaz-Ruiz et al., 2013; Monterroso et al., 2014; Silva et al., 2017). Therefore, it is crucial to understand which factors are the most determinant to the species regional distribution, in order to sustainably manage the landscape.

Our results show a generalised presence of our two-targeted species – red fox and stone marten – in the study area, being present in 99 and 54 of the monitored transects, respectively, and accounting for 73% and 19% of the total recorded data in the Trás-os-Montes region. Both species evidenced distinct ecological strategies and the factors that influence their presence vary between them, but also, in the case of the red fox, the landscape context seemed to influence the presence of this species.

For the red fox, the overall models' results corroborated our Hybrid hypothesis (H4), as its distribution is conditioned by variables associated with different ecological drivers, i.e. factors associated with anthropic disturbance, altitude and landcover patterns, have influence on this species distribution – the altitude, percentage of agriculture, forest and shrubs have a positive effect, while the distance to the nearest urban area and the disturbance associated with the presence of cattle and vehicles, have the opposite effect (see Table IV.3). These results are in agreement with most studies, which indicate that the red fox is a generalist predator that uses resources according to their availability (Scott et al., 2014) – evidence their opportunistic behaviour. Although some of these factors have already been studied and detected, in other areas, as determinants of red fox distribution patterns, when we identify the type of influence that each variables has on the species and try to do a combined interpretation of those variables, the results seem to be contradictory, which makes the pattern difficult to explain – according to our results, the distance to the nearest urban area had a negative influence (i.e., the proximity to an artificial area, increases the probability of the red fox's presence), while the variable percentage of forest had a positive effect. Thus, when the factors were reanalysed separately, taking into account the continuous variable, included in the best models, that showed a bimodal distribution (% of forest within buffer area), it allowed us to identify drivers whose effect would otherwise be masked by the dominant characteristics of this context.

The new results showed that the red fox appears to have two distinct strategies (Figure IV.3): when inhabiting an environment with a high percentage of forest, human activities (e.g. agriculture) have a negative effect; inversely, when in an environment where the forest cover is reduced, the presence of this species is positively influenced by the

agricultural activity and the proximity to urban area, at the same time as the presence of fire and the percentage of scrubs have a negative effect.

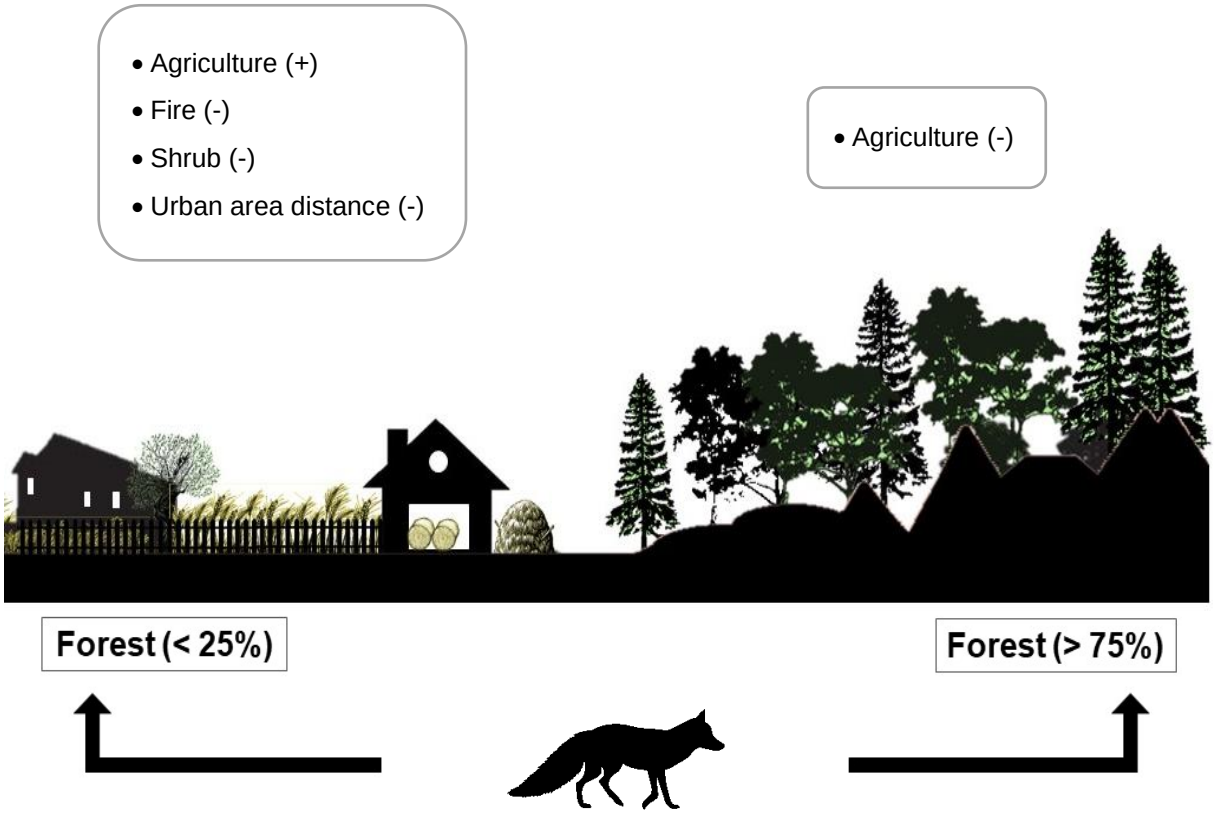

Figure IV.3 – Representation of the two red fox's distribution driver's patterns, resulting from two distinct strategies: when in areas with a low percentage of forest (<25%), agricultural patches and proximity to urban areas have a positive effect; in areas dominated by forest (> 75%), anthropogenic activities (e.g. agriculture) have a negative influence on the presence of this species.

In our study area, when present in environments with high forest cover (>75% forest), the red fox seems to give priority to seeking refuge (Loureiro, 2012), avoiding anthropic disturbance sources, such as agriculture areas. As these animals live in forest areas (closed habitats), where the disturbance is unusual, seems that this species would try to avoid human contact and, therefore, avoid activities that will destabilize the habitat which, in addition to refuge, provides food resources (e.g. rodents; Pereira et al., 2012). On the other hand, those animals inhabiting less forested regions (<25% forest), seem to move closer to settlements and use areas with more agriculture patches, avoiding shrublands. This pattern appears to be driven by the benefits from anthropogenic surplus of resources that are available from nearby humans: food resource – the red fox can benefit significantly from agriculture areas (small mammals, seeds and fruits; Loureiro, 2012), orchards and

market gardens around/ in cities (pets, livestock and human food remains; Contesse et al., 2004; Lewis et al., 1993); and refuges – to ensure their permanent presence, foxes need safe daytime resting places and breeding sites (Baker et al., 2000), so in urban areas they use established gardens (including hedges), old barns and abandoned buildings (Bateman and Fleming, 2012; Gosselink et al., 2007; Scott et al., 2014). In relation to the variables with negative influence: disturbance associated with fire – although the presence of foxes in recent burned areas has been reported (probably due to the small mammals being abundant immediately post disturbance; Fisher and Wilkinson, 2005), after the fire has occurred, some groups of mammals (including canids), only recover their abundance when the affected areas reach the old stage of growth (Fisher and Wilkinson, 2005); percentage of shrublands – one possible explanation for the populations of red fox, that lives in environments with less forest cover, avoiding shrublands areas is that these animals prefer residential green spaces, which, in addition to provide greater opportunities for refuges as well as for food resources (Scott et al., 2014), also allow to avoid competition against other predators (e.g. the absence of a natural predator for the red fox in urban areas in the USA and the UK, results in its lesser predation, compared to rural areas; Bateman and Fleming, 2012; Gosselink et al., 2007). As a consequence of increased food availability in urban habitats, along with protection from predators and avoiding competition with other similar species, the physical condition and the population densities of carnivores are predicted to be favoured. There are already studies that suggest that red fox's populations are denser near urban areas or under human influence (Bateman and Fleming, 2012).

As mentioned before, these very distinct patterns can be explained by the fact that the red fox is a generalist species that has a great adaptability (Díaz-Ruiz et al., 2013), being able to explore several habitats and use very distinct resource types. This high plasticity enables to the species survive in a contrasting environment and exhibit a wide geographic distribution (Loureiro et al., 2012; Palomo et al., 2007). For example, this canid diet is mostly based on insects and small mammal in North Iberia, but on lagomorphs in Southern areas (Díaz-Ruiz et al., 2013), and when in urban areas, as London and Zürich cities, red foxes can survive of anthropogenic foods (wasted fast food; Baker et al., 2000; Contesse et al., 2004).

Regarding the stone marten, it is necessary to be careful when drawing the conclusions from the obtained distribution models, due to the error rates in the morphological identification of scats. However, the results seem to validate our initial hypothesis that the disturbance has a larger influence on the distribution of the species (H1). Its distribution is negatively affected by anthropogenic activities, namely livestock

production. The stone marten is a versatile species (Santos-Reis et al., 2005; Santos and Santos-Reis, 2010) that uses several types of habitats, being associated with transition environments between forest patches and urban areas – mosaic habitat (Santos and Matos, 2012; Santos and Santos-Reis, 2010). However, their populations have been affected, among other factors, by the destruction and reduction of habitats (Santos and Matos, 2012). One of the activities that seems to have a negative effect on the distribution of this species is the presence of cattle, which may have a direct disturbance – the stone marten avoided pastures and only using them during the dispersion activities (Rondinini and Boitani, 2002) –, or represent an indirect disturbance – for example, when the cattle uses watercourses can disturb the riparian galleries, a very important habitat for this mustelid (Santos and Santos-Reis, 2010; Virgós et al., 2012). We also detected a tendency for stone marten's probability of presence being higher nearby settlements, probably due to the availability of food and shelters – in several European cities (e.g. Budapest, Hungary, Luxembourg and Poland), the presence of this species has been reported in areas such as attics, roofs and towers of churches as refuges, and domestic garbage, birds and small mammals, existing in old houses and small gardens, become their food sources (Herr et al., 2009; Tóth and Kis, 2009; Virgós et al., 2012). Another aspect about the stone marten's ecology, when is present in areas where another similar-sized mustelid is present – pine marten –, the first is often more associated with urban areas (Santos and Matos, 2012). Although Monterroso et al. (2016) has reported results that contradict the predominance of pine marten over stone marten, our results may suggest that the presence of stone marten near urban areas may be due, in addition to the greater number of food, to avoid competition with pine marten in higher quality habitats – forests (Balestrieri et al., 2010; Rosellini et al., 2008).

In conclusion, taking all these results into account, we were also able to confirm the opportunistic nature and adaptability of the red fox and the stone marten, which is an advantage when there is an oscillation of resources and disturbances in the environment, characteristics inherent to landscapes shaped by humans, as those present throughout Iberia (Ruiz-Olmo, 2012). This study demonstrates that ecologically similar species may coexist within the same geographic region, being favoured by landscape complexity and habitat diversity. Furthermore, we demonstrated that, at least for the red fox, the landscape context influences the pattern of landscape use. Consequently, considering that populations of the same species are constrained by the same factors, independently of the landscape composition, the efficacy of management measures put in place, to assure the regional survival of a species, may be affected.

Chapter V

5. Final Considerations

The results of this study contribute to improve our knowledge of the dynamics and strategies of some of the most common mesocarnivores in Portugal, and represent an example of how not considering that populations of the same species are influenced by different factors, depending on the composition of the landscape, can lead to ineffectiveness of implemented measures. Although the results have confirmed the adaptability of generalist species – red fox and stone marten – it also shows that it is necessary to continue monitoring and assessing the influence of environmental changes in populations over time, especially after the arise of new disturbance sources (e.g., conversion of traditional agriculture practices into intensive production schemes, habitat fragmentation; Stoate et al., 2009; Verdade et al., 2011) or the increase of the frequency or extension of those already acting (e.g. global warming; Nowark, 2005), is expected. This permanent evaluation is fundamental to understand how species respond to such distinct landscape changes, and identify the ecological strategies developed by those survival populations that allow them to persist in such a changing environment. In this context, and since the study was carried out in a region where the subsistence of the human population is highly dependent on land-use systems oriented for the production of goods (e.g. primary sector – agriculture), we hope that the patterns highlighted by our study can be used by land managers within Trás-os-Montes, in order to reconcile the presence of human activities with the values of biodiversity and conservation, which are crucial in maintaining the structure and functioning of ecosystems.

Furthermore, this thesis explicitly shows the importance of combining methods in the scats identification, especially in areas with a highly diverse mesocarnivore community. The use of multiple methodologies allows the collection of more robust and accurate data that represent, more precisely, the communities present in a given area. We were able to confirm that the use of molecular ecology tools allow a more rigorous identification of the collected scats along the pedestrian transects, increasing the reliability of the data collected and consequently, the results obtained. As an example, if the genetic analysis had not been used in this work (i.e. using only the morphological identification as a criteria), we would have obtained different results regarding the identification of the species and, possibly, by including wrongly identified scats in models, different results and conclusions in the distribution models (as different species have different habitat use patterns; Morrison et al., 2012; Nowark, 2005). This information is extremely important, since the non-rigorous

monitoring can lead to inefficient conservation plans, under or over-estimates of populations and it trends, and inadequate conservation status.

Global climate change and the conservation of the environment have become some of the major concerns for society. Individuals, deliberately or unintentionally, cause deep changes in ecosystems and, consequently, in ecological patterns. Therefore, investigate how these interferences modify the complex structural functioning of an ecosystem, is crucial to mitigate and prevent these changes.

Since these last two years, I have been attending a master's degree in Applied Ecology, where I have learned about the main ecological processes – issues related to the loss and management of Biodiversity, as well as the importance of the ecosystems themselves – so that, in the end, I am allowed to raise awareness and recognize the main adaptations and threats that wildlife face. I manage to contribute to the understanding of the complexity of factors that influence the distribution of two of the predator species with widest distribution in Portugal. Since these species have an important role in the functioning and structure of the ecosystems (key species), any change in their ecological patterns (e.g. distribution range or ecological strategies used to overcome environmental changes) will disturb the entire ecosystem, affecting, at different scales, the conservation of other species. It is these interactions that link several species to one another, making studies, such as this, so important for understanding the complex functioning of the environment and contributing to species conservation.

VI. References

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VII. Appendices

Appendix I

QIAamp® DNA Stool Mini Kit protocol (modified)

Protocol for Isolation of DNA from Stool for Human DNA Analysis

Lysis conditions in this protocol are optimized to increase the ratio of human DNA to non-human DNA. Non-human DNA is not excluded by this procedure.

Important points before starting:

- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on the labels.
- Mix all buffers before use.
- If a precipitate has formed in Buffer ASL or AL, dissolve by incubating at 70°C.
- Prepare a 70°C water bath for use in step 11.
- All centrifugation steps should be carried out at room temperature (15-25°C) at 20,000 x g (~14,000 rpm). Increase the centrifugation time proportionately if your centrifuge cannot provide 20,000 x g (e.g., instead of centrifuging for 5 min at 20,000 x g, centrifuge for 10 min at 10,000 x g).
- The 2 ml tubes used in step 4 should be wide enough to accommodate an InhibitEX Tablet.

1. **Weigh 180-220mg stool in a 2ml microcentrifuge tube (not provided) and place tube on ice.**

   This protocol is optimized for use with 180–220mg stool but can also be used with smaller amounts. There is no need to reduce the amounts of buffers or InhibitEX matrix when using smaller amounts of stool.

   If the sample is liquid, pipet 200µl into the microcentrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2ml microcentrifuge tube on ice.

Note 1: When removing the excrement from the alcohol solution transfer carefully the sample to a Peri dish/ parafilm without transferring much alcohol. The sample doesn't need to be completely dry because the final steps will remove the alcohol left in the sample.

Note 2: When using frozen stool samples, take care that the samples do not thaw until Buffer ASL is added in step 2 to lyse the sample; otherwise the DNA in the sample may

degrade. After addition of Buffer ASL, all following steps can be performed at room temperature (15-25ºC).

2. **Add 1.6ml Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.**
   Following step 2: incubate the homogenate at 56ºC for 1 hour. Vortex every 15 min or keep it on a thermal mixer, 400 rpm.
   Overnight incubation is recommended for old samples.

Note 1: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.

3. **Centrifuge sample at full speed for 1 min to pellet stool particles**.

4. **Pipet 1.4ml of the supernatant into a new 2ml microcentrifuge tube (not provided) and discard the pellet.**

Note: The 2ml tubes used should be wide enough to accommodate an InhibitEX Tablet. Transferring small quantities of pellet material will not affect the procedure.

5. **Add 1 InhibitEX tablet to each sample and vortex immediately and continuously for 1 min or until the tablet is completely suspended. Incubate suspension for 1 min at room temperature (15-25ºC) to allow inhibitors to adsorb to the InhibitEX matrix.**

6. **Centrifuge sample at full speed for 6 min to pellet stool particles and inhibitors bound to InhibitEX.**

Note: When processing more than 12 samples, for this step and step 7 we recommend processing batches of no more than 12 samples each. This is because the pellets formed after centrifugation will break up quickly if the supernatant is not removed immediately.

7. **Immediately after the centrifuge stops, pipet all of the supernatant into a new 1.5ml microcentrifuge tube (not provided) and discard the pellet. Centrifuge the sample at full speed for 3 min.**

Transferring small quantities of pellet material from step 6 will not affect the procedure.

8. **Pipet 25µl Proteinase K into a new 2ml microcentrifuge tube (not provided).**

9. **Pipet 600µl supernatant from step 7 to the 2ml microcentrifuge tub containing proteinase K.**

10. **Add 600µl Buffer AL and vortex for 15 s.**

Note: Do not add proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

11. **Incubate at 70°C for 30 min [vortexing every 10 minutes (optional)].**
    Centrifuge briefly to remove drops from the inside of the tube lid (optional).

12. **Add 600µl of ethanol (96-100%) to the lysate, and mix by vortexing.**
    Centrifuge briefly to remove drops from the inside of the tube lid (optional).

13. **Label the lid of the QIAamp spin columns provided in a 2ml collection tube. Carefully apply 600µl lysate from step 12 to the QIAamp spin column without moistening the rim. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2ml collection tube, and discard the tube containing the filtrate.**
    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

14. **Carefully open the QIAamp spin column, apply a second aliquot of 600µl lysate and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2ml collection tube, and discard the tube containing the filtrate.**
    Close each spin column in order to avoid aerosol formation during centrifugation. If the lysate has not completely passed through the column after centrifugation, centrifuge again until the QIAamp spin column is empty.

15. **Repeat step 14 to load the third aliquot of the lysate onto the spin column.**

16. **Carefully open the QIAamp spin column and add 500µl Buffer AW1. Close the cap and centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2ml collection tube, and discard the collection tube containing the filtrate.**

17. **Carefully open the QIAamp spin column and add 500µl Buffer AW2. Close the cap and centrifuge at full speed for 3 min. Discard the collection tube containing the filtrate.**

Note: Residual Buffer AW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains Buffer AW2, contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp spin column.

18. **Recommended: Place the QIAamp spin column in a new 2ml collection tube (not provided) and discard the collection tube containing the filtrate. Centrifuge at full speed for 1 min.**
    This step helps to eliminate the chance of possible Buffer AW2 carryover.

19. **Transfer the QIAamp spin column into a new, labeled 1.5ml microcentrifuge tube (not provided). Carefully open the QIAamp spin column and pipet 50µl ddH_2O (double-distilled water) directly onto the QIAamp membrane. Close the cap and incubate for 25 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.**

20. **Repeat step 19. Use the same 1.5ml tube from the previous step to elude the second aliquot of 50µl ddH_2O which makes a total of 100µl of DNA extract.**

Note: When using eluates in PCR, for maximum PCR robustness we highly recommend adding BSA to a final concentration of 0.1 µg/µl to the PCR mixture. For maximum PCR specificity, we recommend using HotStarTaq *Plus* DNA Polymerase.

Appendix II

Table VII.1 – Data sheet used during the field sampling for records of mesocarnivore evidences.

<table>
<tr><td colspan="2">Data:_____
Hora:_____</td><td colspan="2">Condições meteorológicas:_____</td></tr>
<tr><td colspan="4">Local</td></tr>
<tr><td colspan="2">Distrito:_____
Sistema de Coordenadas: UTM
Datum: WGS84</td><td colspan="2">Localidade:_____
Quadrícula 10x10:_____ Quadrícula 5x5:_____
Transecto nº (código):_____</td></tr>
<tr><td colspan="2">Habitat (código):_____</td><td colspan="2">Estrato vegetal (altura):_____</td></tr>
<tr><td colspan="4">Ameaças:
□ Incêndios □Contaminação (orgânica/química) □ Outras (infraestruturas, etc.):_____
□ Descarga de entulho □ Veículos recreativos □ Parques Eólicos (dist.):_____
□ Turismo □ Maquinaria □ Presença de Gado (qual?):_____</td></tr>
<tr><td>Indício</td><td>Código</td><td colspan="2">Coordenadas</td></tr>
<tr><td>Espécie: _____
Grau de Conservação:_____</td><td>_____
_____</td><td colspan="2">x:_____
y:_____</td></tr>
</table>

Appendix III

Table VII.2 – All models (up to the first 60), for the hypotheses of disturbance, landcover composition and hybrid, tested for the distribution of red fox. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. LogLik represents the models' log-li kelihood. Within each hypothesis, the models are ranked by the ΔAICc value (delta) – variation between the AICc of each model and the lower AICc detected for models in the same hypothesis. The probability of each model being the best in each hypothesis is presented through the Akaike weight (weight). All the variables used to test the different ecological hypotheses are described in Table IV.1. Intercept is represented by (Int).

Disturbance hypothesis (H1)

```
Global model call: glmer(formula = INDICIO ~ Livestock + Machinery + Debris + Fire + Infra + Pollution + Vehicles +
Dist_urban + Art_area + (1 | CONCELHO),
    data = matrix_raposa, family = binomial)
---
Model selection table
    (Int) Pollution  Debris  Livestock  Fire  Infra    Machinery  Vehicles Dist_urban Art_area df  logLik  AICc delta weight
238 1.6770   20.04           -1.9620  -3.503           0.748800      -2.581 -0.4500            8 -158.072 332.6  0.00  0.104
254 1.7720   20.53           -1.9900  -3.519 -0.81270  0.711300      -2.675 -0.5167            9 -157.159 332.9  0.28  0.090
206 1.6860   20.24           -1.7000  -3.410                         -2.591 -0.4280            7 -159.356 333.0  0.47  0.082
222 1.7890   21.85           -1.7480  -3.437 -0.85530                -2.696 -0.5024            8 -158.328 333.1  0.51  0.081
240 1.6700   20.39  0.128000 -1.9680  -3.500           0.755900      -2.613 -0.4501            9 -158.051 334.6  2.07  0.037
494 1.6760   21.55           -1.9620  -3.502           0.745100      -2.582 -0.4574 -0.025290  9 -158.059 334.7  2.08  0.037
256 1.7640   21.11  0.183700 -2.0000  -3.518 -0.82240  0.721700      -2.721 -0.5178           10 -157.118 334.9  2.32  0.033
510 1.7750   20.90           -1.9900  -3.519 -0.83570  0.713900      -2.678 -0.5107  0.024980 10 -157.147 335.0  2.38  0.032
462 1.6840   21.45           -1.7030  -3.411                         -2.592 -0.4392 -0.038290  8 -159.327 335.1  2.51  0.030
208 1.6820   20.76  0.054940 -1.7010  -3.408                         -2.601 -0.4278            8 -159.352 335.1  2.56  0.029
224 1.7830   21.31  0.107600 -1.7510  -3.435 -0.86010                -2.715 -0.5027            9 -158.313 335.2  2.59  0.029
478 1.7910   21.17           -1.7470  -3.436 -0.87060                -2.697 -0.4985  0.016360  9 -158.322 335.2  2.61  0.028
110 1.6870   19.99           -1.7980  -3.887           0.682100      -2.575                    7 -160.426 335.2  2.61  0.028
78  1.7070   22.05           -1.5740  -3.779                         -2.599                    6 -161.537 335.3  2.75  0.026
174 1.5930   19.83           -1.8620  -3.429           0.729000             -0.4452           7 -160.621 335.6  3.00  0.023
142 1.6020   20.64           -1.6070  -3.337                                -0.4248           6 -161.877 336.0  3.43  0.019
190 1.6730   20.08           -1.8800  -3.441 -0.71920  0.693900             -0.5015           8 -159.884 336.2  3.62  0.017
```

158	1.6900	19.50		-1.6440	-3.359	-0.76220			-0.4883		7	-161.033	336.4	3.83	0.015
126	1.7470	20.95		-1.8030	-3.928	-0.49840	0.660500	-2.625			8	-160.055	336.5	3.97	0.014
94	1.7750	20.06		-1.5900	-3.830	-0.53270		-2.654			7	-161.106	336.5	3.97	0.014
496	1.6700	20.53	0.121300	-1.9680	-3.500		0.752000	-2.612	-0.4568	-0.022970	10	-158.041	336.7	4.17	0.013
512	1.7670	20.99	0.195600	-2.0010	-3.518	-0.85060	0.725500	-2.727	-0.5107	0.029690	11	-157.100	337.0	4.42	0.011
366	1.6880	20.28		-1.8040	-3.878		0.691400	-2.575		0.049840	8	-160.376	337.2	4.61	0.010
464	1.6820	21.14	0.044750	-1.7040	-3.409			-2.600	-0.4389	-0.037460	9	-159.325	337.2	4.61	0.010
112	1.6790	21.04	0.125100	-1.8040	-3.886		0.688200	-2.605			8	-160.406	337.2	4.67	0.010
480	1.7850	20.80	0.114800	-1.7500	-3.434	-0.87830		-2.719	-0.4981	0.019000	10	-158.306	337.3	4.70	0.010
334	1.7080	20.39		-1.5750	-3.771			-2.598		0.035660	7	-161.511	337.4	4.78	0.010
80	1.7030	20.37	0.058200	-1.5760	-3.778			-2.608			7	-161.533	337.4	4.83	0.009
430	1.5920	19.57		-1.8620	-3.429		0.725400		-0.4524	-0.024960	8	-160.608	337.6	5.07	0.008
176	1.5970	19.49	-0.054310	-1.8600	-3.431		0.726000		-0.4451		8	-160.617	337.7	5.09	0.008
398	1.6000	19.70		-1.6100	-3.338				-0.4357	-0.037460	7	-161.850	338.0	5.46	0.007
144	1.6100	19.83	-0.113000	-1.6060	-3.342				-0.4252		7	-161.860	338.1	5.48	0.007
46	1.6080	20.58		-1.7070	-3.815		0.665500				6	-162.980	338.2	5.64	0.006
446	1.6750	20.46		-1.8800	-3.441	-0.73760	0.695900		-0.4967	0.020230	9	-159.875	338.3	5.72	0.006
192	1.6740	20.50	-0.015170	-1.8800	-3.442	-0.71860	0.693100		-0.5014		9	-159.883	338.3	5.73	0.006
382	1.7600	20.78		-1.8120	-3.916	-0.59510	0.672400	-2.635		0.090630	9	-159.888	338.3	5.74	0.006
14	1.6280	21.30		-1.4870	-3.709						5	-164.074	338.3	5.75	0.006
350	1.7860	21.52		-1.5920	-3.816	-0.62140		-2.663		0.080530	8	-160.974	338.4	5.80	0.006
160	1.6950	20.50	-0.076190	-1.6430	-3.362	-0.75950			-0.4882		8	-161.025	338.5	5.91	0.005
414	1.6910	21.52		-1.6440	-3.359	-0.77340			-0.4854	0.012040	8	-161.030	338.5	5.92	0.005
128	1.7380	20.99	0.159700	-1.8110	-3.929	-0.50620	0.668400	-2.663			9	-160.023	338.6	6.01	0.005
96	1.7700	20.88	0.090560	-1.5930	-3.829	-0.53630		-2.670			8	-161.095	338.6	6.05	0.005
368	1.6790	19.90	0.140800	-1.8100	-3.877		0.698800	-2.609		0.052310	9	-160.351	339.2	6.67	0.004
336	1.7030	20.14	0.068830	-1.5770	-3.769			-2.610		0.036830	8	-161.505	339.4	6.87	0.003
30	1.6850	21.43		-1.4990	-3.751	-0.46200					6	-163.740	339.7	7.16	0.003
62	1.6590	20.09		-1.7090	-3.849	-0.42630	0.646000				7	-162.700	339.7	7.16	0.003
432	1.5960	19.97	-0.061850	-1.8600	-3.431		0.721900		-0.4527	-0.026170	9	-160.603	339.7	7.17	0.003
237	1.6630			-1.8910	-3.419		0.857200	-2.681	-0.5170		7	-162.843	340.0	7.45	0.003
400	1.6100	19.82	-0.123700	-1.6080	-3.344				-0.4368	-0.039840	8	-161.829	340.1	7.52	0.002
302	1.6100	19.69		-1.7120	-3.807		0.674400			0.049370	7	-162.930	340.2	7.62	0.002
253	1.7590			-1.9290	-3.440	-0.81290	0.842700	-2.778	-0.5863		8	-161.885	340.2	7.63	0.002
48	1.6130	19.97	-0.057630	-1.7050	-3.817		0.662800				7	-162.975	340.3	7.71	0.002
384	1.7500	20.10	0.199000	-1.8220	-3.918	-0.61050	0.683100	-2.683		0.095260	10	-159.840	340.3	7.77	0.002
270	1.6290	21.00		-1.4880	-3.701					0.035810	6	-164.048	340.3	7.77	0.002
16	1.6370	21.20	-0.110900	-1.4850	-3.712						6	-164.058	340.4	7.79	0.002
448	1.6760	20.96	-0.007522	-1.8800	-3.441	-0.73710	0.695400		-0.4967	0.020050	10	-159.875	340.4	7.84	0.002

```
352 1.7790   20.16  0.123800 -1.5960  -3.815 -0.62990                -2.685          0.083310  9 -160.955 340.4  7.88  0.002
416 1.6960   21.96 -0.072490 -1.6420  -3.361 -0.76920                       -0.4858  0.010350  9 -161.023 340.6  8.01  0.002
205 1.6550                  -1.5600  -3.284                        -2.662 -0.5007            6 -164.639 341.5  8.95  0.001
318 1.6700   19.91          -1.7160  -3.838 -0.51520  0.656500                       0.085010  8 -162.554 341.5  8.96  0.001
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Landcover composition hypothesis (H3)

```
Global model call: glmer(formula = INDICIO ~ Agr_area + Forest + Shrub + Spars_veg + Water +
    (1 | CONCELHO), data = matrix_raposa, family = binomial)
---
Model selection table
   (Int)  Water Agr_area Forest Shrub Spars_veg df logLik  AICc delta weight
32 0.3156 0.7536 1.5780 2.2580 1.68100 0.3323  7 -166.783 347.9  0.00  0.512
31 0.4103        1.4000 1.9630 1.44800 0.2980  6 -168.491 349.2  1.33  0.263
16 0.4695 0.6569 1.2670 1.7860 1.27600         6 -169.199 350.6  2.75  0.130
15 0.4985        1.2030 1.6680 1.19900         5 -170.543 351.3  3.36  0.095
7  0.7024        0.6371 0.7069                 4 -181.262 370.6 22.74  0.000
8  0.7148 0.5684 0.6465 0.7367                 5 -180.443 371.1 23.16  0.000
23 0.6967        0.6555 0.7264         0.1772  5 -180.566 371.3 23.41  0.000
24 0.7089 0.5844 0.6655 0.7571         0.1801  6 -179.717 371.7 23.78  0.000
13 0.7186               0.6721 0.37470         4 -186.629 381.4 33.48  0.000
14 0.7780 0.6577        0.6852 0.37110         5 -186.141 382.5 34.56  0.000
29 0.7135               0.6864 0.38190 0.1360  5 -186.191 382.6 34.66  0.000
30 0.7752 0.6768        0.6995 0.37830 0.1364  6 -185.696 383.6 35.74  0.000
5  0.7346               0.4734                 3 -188.811 383.7 35.79  0.000
3  0.7063        0.4161                        3 -189.132 384.3 36.43  0.000
6  0.7868 0.6343        0.4897                 4 -188.291 384.7 36.80  0.000
21 0.7317               0.4816         0.1302  4 -188.439 385.0 37.10  0.000
19 0.7041        0.4238                0.1357  4 -188.758 385.6 37.73  0.000
11 0.7035        0.4364        0.13440         4 -188.759 385.6 37.74  0.000
4  0.7262 0.3150 0.4145                        4 -188.890 385.9 38.00  0.000
22 0.7857 0.6499        0.4981         0.1308  5 -187.912 386.0 38.10  0.000
27 0.7008        0.4442        0.13490 0.1346  5 -188.383 386.9 39.04  0.000
20 0.7244 0.3194 0.4222                0.1364  5 -188.512 387.2 39.30  0.000
```

```
12 0.7230 0.2876 0.4336        0.12690         5 -188.558 387.3 39.39  0.000
28 0.7207 0.2917 0.4414        0.12740 0.1352  6 -188.178 388.6 40.71  0.000
1  0.7315                                      2 -192.874 389.8 41.88  0.000
17 0.7302                              0.1120  3 -192.617 391.3 43.40  0.000
2  0.7697 0.4123                               3 -192.628 391.3 43.43  0.000
9  0.7318                      0.06060         3 -192.796 391.7 43.76  0.000
18 0.7694 0.4192                       0.1124  4 -192.369 392.9 44.96  0.000
25 0.7303                      0.05987 0.1109  4 -192.542 393.2 45.30  0.000
10 0.7698 0.4017               0.05390         4 -192.567 393.3 45.35  0.000
26 0.7692 0.4088               0.05312 0.1115  5 -192.310 394.8 46.90  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Hybrid hypothesis (H4)

```
Global model call: glmer(formula = INDICIO ~ Livestock + Vehicles + Dist_urban + Agr_area + Fire + Forest +
      Shrub + Alt + (1 | CONCELHO), data = matrix_raposa, family = binomial)
---
Model selection table
    (Int) Agr_area  Alt  Livestock  Fire   Vehicles Dist_urban Forest  Shrub   df  logLik  AICc delta weight
248 1.2540 1.49100 0.6026 -1.9740             -3.248 -0.6674 1.9930  1.595000  9 -148.499 315.5  0.00  0.580
256 1.3260 1.39100 0.5778 -1.9640 -0.6412      -3.228 -0.6226 1.8640  1.446000 10 -148.254 317.2  1.63  0.256
232 1.1440 1.40000 0.5643 -1.8230                    -0.6692 1.9620  1.563000  8 -152.202 320.8  5.30  0.041
246 1.1930 1.43500        -1.8590             -3.057 -0.6286 1.9240  1.410000  8 -152.533 321.5  5.96  0.029
224 1.4210 1.38500 0.5216 -1.7930 -1.3130      -3.287         1.6720  1.271000  9 -151.769 322.1  6.54  0.022
216 1.2770 1.61500 0.5717 -1.7970             -3.361         1.9390  1.581000  8 -152.909 322.2  6.71  0.020
254 1.3190 1.28500        -1.8500 -0.9828      -3.043 -0.5600 1.7320  1.198000  9 -151.880 322.3  6.76  0.020
240 1.2220 1.30000 0.5379 -1.8100 -0.6714            -0.6220 1.8310  1.412000  9 -151.926 322.4  6.85  0.019
230 1.1080 1.35700        -1.7370                    -0.6361 1.9020  1.394000  7 -155.918 326.2 10.63  0.003
222 1.3900 1.30500        -1.7040 -1.5600      -3.149         1.5850  1.081000  8 -154.941 326.3 10.78  0.003
238 1.2270 1.21400        -1.7280 -0.9820            -0.5687 1.7170  1.189000  8 -155.254 326.9 11.40  0.002
208 1.3200 1.29400 0.4886 -1.6470 -1.3440                    1.6390  1.239000  8 -155.568 327.6 12.03  0.001
214 1.2090 1.56100        -1.6900             -3.188         1.8850  1.418000  7 -156.720 327.8 12.24  0.001
200 1.1730 1.51800 0.5345 -1.6440                            1.9020  1.545000  7 -156.793 327.9 12.38  0.001
128 1.6720 0.53620 0.3810 -1.7880 -2.8500      -3.108 -0.4649 0.6371            9 -155.311 329.2 13.62  0.001
126 1.6290 0.57270        -1.7350 -2.7300      -3.018 -0.4569 0.6972            8 -157.298 331.0 15.49  0.000
206 1.3040 1.23400        -1.5820 -1.5680                    1.5660  1.071000  7 -158.497 331.3 15.79  0.000
```

96	1.7150	0.61180	0.3764	-1.6840	-3.1780	-3.179		0.6065		8	-157.474	331.4	15.84	0.000
198	1.1220	1.48900		-1.5660				1.8660	1.406000	6	-160.323	332.9	17.36	0.000
94	1.6650	0.64850		-1.6290	-3.0790	-3.110		0.6622		7	-159.453	333.2	17.70	0.000
112	1.5680	0.46650	0.3603	-1.6530	-2.8050		-0.4729	0.6248		8	-158.818	334.1	18.53	0.000
127	1.6460		0.4362	-1.5990	-3.1680	-2.703	-0.5863	0.4103		8	-159.125	334.7	19.14	0.000
110	1.5380	0.50600		-1.6160	-2.7000		-0.4680	0.6835		7	-160.659	335.7	20.11	0.000
64	1.7250	0.31300	0.4725	-1.7430	-3.2600	-3.126	-0.4267			8	-159.689	335.8	20.27	0.000
80	1.6140	0.54350	0.3575	-1.5490	-3.1450			0.5928		7	-161.129	336.6	21.05	0.000
255	1.6220		0.4503	-1.5900	-3.0130	-2.670	-0.6105	0.4682	0.092460	9	-159.055	336.6	21.11	0.000
63	1.6970		0.4885	-1.6280	-3.3980	-2.799	-0.5177			7	-161.240	336.8	21.28	0.000
32	1.7490	0.39360	0.4573	-1.6320	-3.5160	-3.199				7	-161.635	337.6	22.07	0.000
192	1.7320	0.28620	0.4537	-1.7330	-3.3920	-3.109	-0.4212		-0.086890	9	-159.597	337.7	22.20	0.000
125	1.6090			-1.5390	-3.0430	-2.565	-0.5903	0.4645		7	-161.790	337.9	22.38	0.000
191	1.7220		0.4456	-1.6330	-3.6650	-2.839	-0.4888		-0.189100	8	-160.753	337.9	22.40	0.000
111	1.5610		0.4131	-1.5060	-3.0940		-0.5794	0.4238		7	-161.831	338.0	22.46	0.000
78	1.5760	0.58390		-1.5080	-3.0590			0.6468		6	-162.982	338.2	22.68	0.000
160	1.7610	0.35820	0.4358	-1.6250	-3.6800	-3.177			-0.109700	8	-161.485	339.4	23.86	0.000
239	1.5280		0.4345	-1.4950	-2.8680		-0.6154	0.5098	0.138200	8	-161.669	339.8	24.23	0.000
95	1.6840		0.4430	-1.4200	-3.6550	-2.682		0.3302		7	-162.805	339.9	24.41	0.000
253	1.6110			-1.5400	-3.0580	-2.569	-0.5881	0.4590	-0.008846	8	-161.790	340.0	24.47	0.000
62	1.6770	0.33060		-1.6750	-3.1430	-3.021	-0.4033			7	-162.846	340.0	24.49	0.000
47	1.6050		0.4643	-1.5290	-3.3170		-0.5064			6	-164.159	340.6	25.03	0.000
48	1.6180	0.24600	0.4485	-1.6090	-3.1990		-0.4334			7	-163.149	340.6	25.10	0.000
31	1.7180		0.4776	-1.4500	-3.7830	-2.764				6	-164.295	340.8	25.30	0.000
109	1.5360			-1.4600	-2.9870		-0.5875	0.4747		6	-164.302	340.9	25.32	0.000
159	1.7590		0.4276	-1.4800	-4.1080	-2.830			-0.248200	7	-163.415	341.2	25.63	0.000
190	1.6980	0.27380		-1.6590	-3.4280	-3.000	-0.3971		-0.184900	8	-162.397	341.2	25.69	0.000
189	1.6980			-1.5720	-3.6900	-2.744	-0.4663		-0.280900	7	-163.487	341.3	25.77	0.000
61	1.6550			-1.5600	-3.2840	-2.662	-0.5007			6	-164.639	341.5	25.99	0.000
244	0.4454	1.15000	0.4895			-2.416	-0.4744	1.7380	1.321000	8	-162.578	341.6	26.05	0.000
30	1.6940	0.40930		-1.5690	-3.4140	-3.116				6	-164.684	341.6	26.08	0.000
175	1.6270		0.4265	-1.5320	-3.5530		-0.4816		-0.168800	7	-163.758	341.8	26.31	0.000
223	1.7060		0.4305	-1.4370	-3.7840	-2.718		0.2789	-0.086720	8	-162.736	341.9	26.36	0.000
176	1.6260	0.21880	0.4296	-1.5990	-3.3370		-0.4280		-0.091100	8	-163.045	342.5	26.98	0.000
158	1.7210	0.34740		-1.5590	-3.7150	-3.091			-0.197100	7	-164.163	342.7	27.12	0.000
16	1.6450	0.32810	0.4349	-1.4990	-3.4680					6	-165.223	342.7	27.16	0.000
252	0.5382	1.04200	0.4633		-0.7746	-2.409	-0.4220	1.5930	1.160000	9	-162.162	342.9	27.33	0.000

```
237 1.5270                -1.4570 -2.9250             -0.5971 0.4987  0.038170  7 -164.288 342.9 27.37  0.000
79  1.6060         0.4238 -1.3360 -3.5860                     0.3429            6 -165.514 343.3 27.74  0.000
93  1.6330                -1.3470 -3.5590      -2.571         0.3727            6 -165.677 343.6 28.07  0.000
228 0.4128 1.10800 0.4769                             -0.4900 1.7330  1.319000  7 -164.801 343.9 28.40  0.000
157 1.7270                -1.4230 -4.1310      -2.761                -0.328900  6 -166.052 344.4 28.82  0.000
144 1.6590 0.29310 0.4138 -1.4930 -3.6360                       -0.112300  7 -165.059 344.5 28.92  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Appendix IV

Table VII.3 – All models (up to the first 60), for the hypotheses of disturbance, landcover composition and hybrid, tested for the distribution of red fox when the percentage of forest in buffer area is less than 25%. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. LogLik represents the models' log-likelihood. Within each hypothesis, the models are ranked by the ΔAICc value (delta) – variation between the AICc of each model and the lower AICc detected for models in the same hypothesis. The probability of each model being the best in each hypothesis is presented through the Akaike weight (weight). All the variables used to test the different ecological hypotheses are described in Table IV.1. Intercept is represented by (Int).

Disturbance hypothesis (H1)

```
Global model call: glmer(formula = INDICIO ~ Livestock + Machinery + Debris + Fire + Infra + Pollution + Vehicles +
    Dist_urban + Art_area + (1 | CONCELHO),
    data = matrix_raposa_FLOR25, family = binomial)
---
Model selection table
       (Int) Pollution  Debris Livestock   Fire   Infra    Machinery   Vehicles Dits_urban Art_area df  logLik  AICc delta weight
137  0.599200                            -2.438                             -0.9930             4 -62.451 133.2  0.00  0.058
141  0.940100                  -0.681600 -2.700                             -1.0740             5 -61.717 133.9  0.70  0.041
201  0.652400                            -2.472                    -13.51 -0.9585             5 -61.809 134.1  0.89  0.037
138  0.595600   17.04                    -2.445                             -0.9261             5 -61.884 134.3  1.04  0.034
142  0.996900   18.22          -0.809200 -2.763                             -0.9940             6 -60.865 134.4  1.21  0.032
205  1.036000                  -0.742200 -2.765                    -14.64 -1.0380             6 -60.933 134.6  1.34  0.030
206  1.096000   18.29          -0.874900 -2.832                    -18.83 -0.9534             7 -60.024 135.0  1.77  0.024
139  0.640400         -0.65590           -2.490                             -1.0080             5 -62.251 135.0  1.77  0.024
202  0.648200   17.06                    -2.480                    -17.06 -0.8905             6 -61.225 135.2  1.93  0.022
169  0.641400                            -2.453           -0.33310          -1.0390             5 -62.333 135.2  1.93  0.022
153  0.569100                            -2.422  0.216600                   -0.9707             5 -62.421 135.3  2.11  0.020
393  0.599400                            -2.438                             -0.9925  0.0016960  5 -62.451 135.4  2.17  0.020
170  0.664500   17.46                    -2.477           -0.55290          -0.9857             6 -61.580 135.9  2.64  0.015
203  0.695500         -0.66940           -2.524                    -13.70 -0.9732             6 -61.600 135.9  2.68  0.015
143  0.948600         -0.45690 -0.639800 -2.718                             -1.0770             6 -61.623 136.0  2.72  0.015
233  0.700200                            -2.489           -0.36310 -14.26 -1.0110             6 -61.668 136.0  2.81  0.014
```

140	0.634400	16.88	-0.63000		-2.494				-0.9424		6	-61.698	136.1	2.87	0.014
397	0.938900			-0.686700	-2.704				-1.0850	-0.0290100	6	-61.713	136.1	2.90	0.014
173	0.939800			-0.665700	-2.697		-0.05986		-1.0810		6	-61.714	136.1	2.90	0.014
157	0.952500			-0.690500	-2.708	-0.055120			-1.0800		6	-61.716	136.1	2.91	0.013
217	0.624100				-2.457	0.201400		-14.11	-0.9376		6	-61.782	136.3	3.04	0.013
457	0.653100				-2.471			-14.85	-0.9565	0.0058950	6	-61.809	136.3	3.09	0.012
154	0.555800	16.13			-2.426	0.287900			-0.8929		6	-61.829	136.4	3.13	0.012
394	0.598200	17.15			-2.444				-0.9179	0.0233900	6	-61.882	136.5	3.24	0.011
174	1.005000	17.75		-0.749700	-2.762		-0.30130		-1.0180		7	-60.782	136.5	3.29	0.011
144	1.003000	17.56	-0.38170	-0.772500	-2.775				-0.9990		7	-60.799	136.6	3.32	0.011
207	1.045000		-0.45550	-0.701300	-2.782			-14.58	-1.0410		7	-60.839	136.6	3.40	0.011
398	0.996700	16.95		-0.809800	-2.763				-0.9953	-0.0035320	7	-60.865	136.7	3.45	0.010
158	0.998700	17.45		-0.810500	-2.764	-0.008134			-0.9950		7	-60.865	136.7	3.45	0.010
234	0.724300	17.40			-2.516		-0.58770	-16.15	-0.9564		7	-60.881	136.7	3.48	0.010
221	1.062000			-0.760700	-2.780	-0.109200		-14.08	-1.0500		7	-60.926	136.8	3.57	0.010
237	1.036000			-0.725100	-2.762		-0.06615	-14.14	-1.0460		7	-60.929	136.8	3.58	0.010
461	1.035000			-0.746900	-2.768			-15.71	-1.0480	-0.0264500	7	-60.930	136.8	3.58	0.010
171	0.701600		-0.77910		-2.521		-0.42830		-1.0700		6	-62.065	136.8	3.61	0.010
204	0.688800	16.22	-0.64260		-2.528			-15.22	-0.9069		7	-61.030	137.0	3.78	0.009
238	1.107000	17.33		-0.815600	-2.834		-0.31560	-16.08	-0.9812		8	-59.931	137.1	3.87	0.008
155	0.609400		-0.65730		-2.475	0.222200			-0.9853		6	-62.220	137.2	3.92	0.008
208	1.103000	18.65	-0.37670	-0.839300	-2.843			-18.60	-0.9585		8	-59.959	137.2	3.92	0.008
395	0.638700		-0.66210		-2.492				-1.0140	-0.0180600	6	-62.250	137.2	3.98	0.008
222	1.111000	16.85		-0.885500	-2.841	-0.062350		-17.60	-0.9604		8	-60.022	137.3	4.05	0.008
462	1.096000	19.22		-0.874900	-2.832			-19.46	-0.9532	0.0005711	8	-60.024	137.3	4.05	0.008
185	0.607600				-2.435	0.253300	-0.34850		-1.0150		6	-62.292	137.3	4.06	0.008
218	0.609900	17.70			-2.461	0.274100		-17.29	-0.8585		7	-61.173	137.3	4.07	0.008
425	0.641200				-2.453		-0.33320		-1.0400	-0.0015490	6	-62.333	137.4	4.14	0.007
458	0.651400	17.76			-2.478			-17.45	-0.8806	0.0281800	7	-61.221	137.4	4.16	0.007
172	0.727400	17.92	-0.81940		-2.548		-0.66320		-1.0190		7	-61.285	137.5	4.29	0.007
409	0.567000				-2.423	0.221800			-0.9739	-0.0114300	6	-62.420	137.6	4.32	0.007
235	0.764100		-0.80350		-2.559		-0.46170	-13.15	-1.0430		7	-61.383	137.7	4.49	0.006
186	0.615400	16.74			-2.452	0.384600	-0.59480		-0.9430		7	-61.485	137.9	4.69	0.006
219	0.666400		-0.67040		-2.509	0.206000		-14.21	-0.9521		7	-61.572	138.1	4.87	0.005
426	0.667600	16.97			-2.475		-0.55280		-0.9762	0.0262200	7	-61.577	138.1	4.88	0.005
459	0.694200		-0.67430		-2.526			-14.12	-0.9780	-0.0141100	7	-61.599	138.2	4.92	0.005
175	0.949000		-0.51490	-0.594900	-2.714		-0.15130		-1.0930		7	-61.603	138.2	4.93	0.005
399	0.947100		-0.46820	-0.645600	-2.725				-1.0920	-0.0405100	7	-61.615	138.2	4.95	0.005
159	0.955100		-0.45480	-0.644600	-2.722	-0.029270			-1.0800		7	-61.623	138.2	4.97	0.005

```
249  0.667700                          -2.472  0.241400 -0.37790      -14.38 -0.9871             7 -61.630 138.2  4.98  0.005
156  0.593900   16.61 -0.63040         -2.474  0.291700                      -0.9093             7 -61.641 138.2  5.00  0.005
489  0.700500                          -2.489           -0.36290      -14.61 -1.0100  0.0023030  7 -61.668 138.3  5.06  0.005
396  0.634800   16.40 -0.62860         -2.493                                -0.9409  0.0041490  7 -61.698 138.4  5.12  0.004
236  0.791100   17.34 -0.84550         -2.589           -0.70250      -15.27 -0.9907             8 -60.566 138.4  5.14  0.004
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Landcover composition hypothesis (H3)

```
Global model call: glmer(formula = INDICIO ~ Agr_area + Shrub + Spars_veg + Water + (1 |
    CONCELHO), data = matrix_raposa_FLOR25, family = binomial)
---
Model selection table
     (Int) Water Agr_area Shrub Spars_veg df logLik AICc delta weight
8   0.02445 -2.770 1.767 -0.6322         5 -60.507 131.5  0.00  0.270
16 -5.91200 -6.501 1.706 -0.5815 -46.67  6 -59.579 131.9  0.35  0.226
7   0.12820        1.596 -0.6125         4 -62.090 132.5  1.00  0.164
15 -3.32100        1.540 -0.5620 -28.78  5 -61.321 133.1  1.63  0.119
12 -3.61800 -2.874 1.464         -30.86  5 -61.759 134.0  2.50  0.077
4   0.12890 -2.182 1.501                 4 -63.103 134.5  3.02  0.060
11 -2.89400        1.320         -25.84  4 -63.389 135.1  3.59  0.045
3   0.19530        1.354                 3 -64.578 135.4  3.84  0.040
9  -3.13300                      -28.68  3 -73.133 152.5 20.95  0.000
13 -3.12700              -0.2320 -28.46  4 -72.662 153.7 22.14  0.000
10 -3.31400 -1.929               -28.97  4 -72.808 153.9 22.43  0.000
1   0.28000                              2 -74.926 154.0 22.43  0.000
5   0.25790              -0.2769         3 -74.245 154.7 23.17  0.000
14 -3.37800 -1.732       -0.2263 -29.48  5 -72.354 155.2 23.69  0.000
2   0.16660 -1.462                       3 -74.709 155.6 24.10  0.000
6   0.15280 -1.361       -0.2722         4 -74.047 156.4 24.91  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Hybrid hypothesis (H4)

```
Global model call: glmer(formula = INDICIO ~ Agr_area + Shrub + Dist_urban + Fire +
    (1 | CONCELHO), data = matrix_raposa_FLOR25, family = binomial)
---
Model selection table
   (Int)  Agr_area Fire  Dist_urban Shrub df logLik  AICc delta weight
16 0.4478 1.0030  -3.089 -1.0470 -0.9459  6 -54.517 121.7  0.00  0.762
12 0.5365 1.3040  -3.222         -0.8539  5 -57.867 126.2  4.49  0.081
14 0.0189 1.2310         -1.1570 -0.7511  5 -57.971 126.4  4.70  0.073
15 0.6303         -3.468 -1.2950 -0.8245  5 -58.400 127.3  5.56  0.047
8  0.4573 0.8042  -1.973 -0.8033          5 -59.349 129.2  7.46  0.018
6  0.1534 1.0110         -0.9131          4 -61.310 131.0  9.21  0.008
10 0.1282 1.5960                 -0.6125  4 -62.090 132.5 10.77  0.003
4  0.5003 1.0650  -2.185                   4 -62.123 132.6 10.83  0.003
7  0.5992         -2.438 -0.9930          4 -62.451 133.2 11.49  0.002
2  0.1953 1.3540                          3 -64.578 135.4 13.61  0.001
13 0.1415                -1.5060 -0.5644  4 -64.143 136.6 14.87  0.000
5  0.2183                -1.2520          3 -66.283 138.8 17.02  0.000
11 0.7611         -3.737         -0.5916  4 -65.389 139.1 17.37  0.000
3  0.7219         -3.002                  3 -68.094 142.4 20.64  0.000
1  0.2800                                 2 -74.926 154.0 32.21  0.000
9  0.2579                        -0.2769  3 -74.245 154.7 32.94  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Appendix V

Table VII.4 – All models (up to the first 60), for the hypotheses of disturbance, landcover composition and hybrid, tested for the distribution of red fox when the percentage of forest in buffer area is greater than 75%. The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. LogLik represents the models' log-likelihood. Within each hypothesis, the models are ranked by the ΔAICc value (delta) – variation between the AICc of each model and the lower AICc detected for models in the same hypothesis. The probability of each model being the best in each hypothesis is presented through the Akaike weight (weight). All the variables used to test the different ecological hypotheses are described in Table IV.1. Intercept is represented by (Int).

Disturbance hypothesis (H1)

```
Global model call: glmer(formula = INDICIO ~ Livestock + Machinery + Debris + Fire + Infra + Pollution +
    Vehicles + Dist_urban + Art_area + (1 | CONCELHO), data = matrix_raposa_FLOR75, family = binomial)
---
Model selection table
      (Int) Pollution Debris  Livestock Fire   Infra   Machinery   Dist_urban Vehicles Art_area df logLik  AICc delta weight
109 2.41000                    -8.118  -3.942           5.596000      -4.815                     6 -36.855  86.7  0.00  0.116
110 2.25500  18.350            -7.745  -3.862           5.468000      -4.551                     7 -36.453  88.2  1.54  0.054
45  1.91800                    -6.471  -3.466           4.325000                                 5 -38.982  88.7  1.97  0.043
111 2.33800          0.790800  -8.230  -4.006           5.652000      -5.159                     7 -36.781  88.9  2.19  0.039
237 2.42900                    -8.019  -4.081           5.504000      -4.774  0.156000           7 -36.825  89.0  2.28  0.037
365 2.84700                    -8.085  -3.934           5.580000      -4.802            3.0620   7 -36.843  89.0  2.32  0.036
125 2.40100                    -8.177  -3.922  0.25590  5.670000      -4.828                     7 -36.848  89.0  2.33  0.036
46  1.81200  12.930            -6.304  -3.453           4.419000                                 6 -38.275  89.5  2.84  0.028
101 1.66600                    -7.578                   4.895000      -4.156                     5 -39.486  89.7  2.97  0.026
112 2.19600  13.080  0.697600  -7.861  -3.928           5.535000      -4.866                     8 -36.393  90.5  3.81  0.017
126 2.22500  20.880            -7.859  -3.820  0.57880  5.643000      -4.563                     8 -36.414  90.5  3.85  0.017
238 2.27400  25.690            -7.655  -3.986           5.382000      -4.514  0.140400           8 -36.428  90.6  3.88  0.017
366 2.50400  13.410            -7.703  -3.852           5.449000      -4.533            1.8030   8 -36.438  90.6  3.90  0.016
173 1.93900                    -6.335  -3.620           4.194000              0.177600           6 -38.938  90.9  4.16  0.014
61  1.92000                    -6.646  -3.465  0.47360  4.535000                                 6 -38.949  90.9  4.19  0.014
301 2.51900                    -6.431  -3.456           4.308000                      4.1960     6 -38.960  90.9  4.21  0.014
47  1.90900          0.131900  -6.512  -3.485           4.363000                                 6 -38.979  90.9  4.25  0.014
```

37	1.26100			-5.798			3.414000				4	-41.342	91.1	4.45	0.013
102	1.52800	15.860		-7.187			4.742000	-3.886			6	-39.083	91.1	4.46	0.013
239	2.35500		0.803400	-8.117	-4.150		5.545000	-5.116	0.162400		8	-36.748	91.2	4.52	0.012
367	2.38200		0.737500	-7.978	-3.940		5.454000	-5.032		0.7181	8	-36.774	91.3	4.58	0.012
127	2.33700		0.772200	-8.251	-3.999	0.09074	5.680000	-5.157			8	-36.780	91.3	4.59	0.012
493	3.79300			-7.978	-4.076		5.483000	-4.757	0.160600	9.3680	8	-36.811	91.3	4.65	0.011
253	2.42100			-8.076	-4.059	0.25030	5.575000	-4.787	0.154900		8	-36.818	91.4	4.66	0.011
381	2.76500			-8.144	-3.914	0.25810	5.655000	-4.815		2.5650	8	-36.836	91.4	4.70	0.011
62	1.80800	15.220		-6.671	-3.464	0.93610	4.893000				7	-38.139	91.6	4.91	0.010
103	1.58900		0.935100	-7.760			4.968000	-4.592			6	-39.354	91.7	5.00	0.010
229	1.65400			-7.661			4.989000	-4.214	-0.268100		6	-39.381	91.7	5.05	0.009
174	1.83100	16.480		-6.187	-3.581		4.303000		0.150200		7	-38.243	91.8	5.12	0.009
302	2.27500	23.120		-6.254	-3.441		4.400000			3.2800	7	-38.247	91.8	5.13	0.009
48	1.80400	25.390	0.126700	-6.344	-3.472		4.457000				7	-38.273	91.9	5.18	0.009
117	1.65800			-7.720		0.50340	5.057000	-4.200			6	-39.460	91.9	5.21	0.009
357	2.18500			-7.549			4.882000	-4.145		3.5990	6	-39.475	91.9	5.24	0.008
38	1.16500	27.290		-5.636			3.506000				5	-40.674	92.0	5.35	0.008
13	1.55400			-3.184	-2.782						4	-41.985	92.4	5.74	0.007
240	2.20700	8.442	0.701500	-7.753	-4.052		5.427000	-4.828	0.142100		9	-36.366	92.9	6.21	0.005
128	2.18200	13.220	0.603500	-7.945	-3.891	0.45390	5.676000	-4.838			9	-36.370	92.9	6.22	0.005
368	2.35900	9.455	0.687500	-7.808	-3.914		5.507000	-4.841		1.2260	9	-36.379	92.9	6.24	0.005
77	1.69900			-3.362	-3.021			-2.396			5	-41.128	92.9	6.26	0.005
254	2.24300	16.670		-7.768	-3.935	0.56070	5.550000	-4.527	0.131800		9	-36.391	93.0	6.26	0.005
382	2.47600	15.840		-7.816	-3.809	0.58330	5.624000	-4.545		1.8260	9	-36.397	93.0	6.28	0.005
494	2.57700	48.280		-7.604	-3.980		5.356000	-4.493	0.146100	2.1820	9	-36.411	93.0	6.30	0.005
189	1.93800			-6.497	-3.609	0.44830	4.388000		0.170200		7	-38.909	93.1	6.45	0.005
429	2.36400			-6.281	-3.617		4.170000		0.185500	3.0030	7	-38.912	93.1	6.45	0.005
317	2.60900			-6.605	-3.456	0.47460	4.519000			4.7930	7	-38.927	93.2	6.49	0.005
175	1.93000		0.134700	-6.372	-3.639		4.228000		0.178300		7	-38.935	93.2	6.50	0.004
63	1.91500		0.074720	-6.669	-3.477	0.46480	4.556000				7	-38.948	93.2	6.53	0.004
165	1.25800			-5.901			3.529000		-0.214000		5	-41.266	93.2	6.53	0.004
303	2.72700		0.126100	-6.470	-3.475		4.345000			5.6680	7	-38.958	93.2	6.55	0.004
230	1.51400	29.190		-7.274			4.846000	-3.945	-0.278300		7	-38.966	93.3	6.56	0.004
104	1.46500	29.620	0.818500	-7.361			4.820000	-4.279			7	-38.978	93.3	6.59	0.004
53	1.25600			-5.944		0.47100	3.592000				5	-41.310	93.3	6.62	0.004
293	1.86100			-5.770			3.408000			4.1530	5	-41.323	93.3	6.65	0.004
118	1.50000	14.960		-7.371		0.77310	4.989000	-3.925			7	-39.013	93.3	6.66	0.004
39	1.24900		0.128800	-5.823			3.433000				5	-41.339	93.4	6.68	0.004
358	1.66600	8.991		-7.114			4.699000	-3.856		1.0750	7	-39.068	93.5	6.77	0.004

```
495 3.48200          0.797200  -8.077  -4.145          5.525000      -5.098  0.166800  7.7480  9 -36.735  93.6  6.95  0.004
255 2.35800          0.786200  -8.135  -4.143  0.07407 5.568000      -5.109  0.161100          9 -36.747  93.7  6.98  0.004
383 3.78300          0.765700  -8.219  -3.991  0.09441 5.666000      -5.142            9.9120  9 -36.768  93.7  7.02  0.003
509 3.53400                    -8.035  -4.054  0.25250 5.554000      -4.770  0.159500  7.6720  9 -36.805  93.8  7.09  0.003
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Landcover composition hypothesis (H3)

```
Global model call: glmer(formula = INDICIO ~ Agr_area + Shrub + Spars_veg + (1 | CONCELHO),
    data = matrix_raposa_FLOR75, family = binomial)
---
Model selection table
   (Int) Agr_area Shrub Spars_veg df  logLik  AICc delta weight
4 0.06091 -2.156 0.9359           4 -36.567  81.6  0.00  0.533
8 0.04144 -2.180 0.9633 -0.31270  5 -36.391  83.5  1.88  0.208
2 0.21820 -2.023                  3 -38.695  83.7  2.07  0.189
6 0.21200 -2.042        -0.20210  4 -38.593  85.6  4.05  0.070
3 0.05802        1.0270           3 -47.519 101.3 19.72  0.000
7 0.04769        1.0590 -0.16340  4 -47.438 103.3 21.74  0.000
1 0.13710                         2 -50.573 105.3 23.69  0.000
5 0.13660               -0.01781  3 -50.572 107.4 25.83  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Hybrid hypothesis (H4)

```
Global model call: glmer(formula = INDICIO ~ Livestock + Machinery + Fire + Alt +
    Agr_area + Shrub + (1 | CONCELHO), data = matrix_raposa_FLOR75, family = binomial)
---
Model selection table
    (Int) Agr_area  Alt  Livestock Fire  Machinery Shrub  df logLik  AICc delta weight
46 1.88600 -2.307         -3.200  -5.197          1.1370  6 -31.501  76.0  0.00  0.157
62 2.11000 -2.012         -5.423  -5.022  3.09300 1.1810  7 -30.795  76.9  0.93  0.098
48 1.86200 -2.227 0.5399  -3.062  -4.978          1.2280  7 -30.919  77.2  1.18  0.087
```

64	2.05500	-1.846	0.7340	-5.560	-4.667	3.66500	1.2900	8	-29.809	77.3	1.35	0.080
14	1.90600	-2.294		-2.635	-5.087			5	-33.341	77.4	1.39	0.078
30	1.97400	-2.088		-4.401	-4.944	2.60000		6	-32.430	77.8	1.86	0.062
42	0.71970	-2.476			-4.634		0.8795	5	-33.876	78.4	2.46	0.046
32	1.89700	-1.959	0.5641	-4.270	-4.743	2.92900		7	-31.712	78.7	2.76	0.039
44	0.75800	-2.385	0.6136		-4.424		0.9685	6	-32.930	78.8	2.86	0.038
16	1.87000	-2.238	0.3928	-2.428	-4.951			6	-32.972	78.9	2.94	0.036
38	1.12900	-1.903		-3.049			1.2030	5	-34.204	79.1	3.12	0.033
10	0.92220	-2.285			-4.459			4	-35.395	79.2	3.26	0.031
54	1.31400	-1.650		-5.366		2.89300	1.3570	6	-33.325	79.6	3.65	0.025
56	1.31500	-1.507	0.7613	-5.460		3.28600	1.5190	7	-32.167	79.7	3.67	0.025
40	1.13400	-1.827	0.5588	-2.909			1.3040	6	-33.494	80.0	3.98	0.021
12	1.00300	-2.268	0.4884		-4.445			5	-34.717	80.1	4.14	0.020
58	0.78830	-2.475			-4.695	-0.28590	0.8979	6	-33.851	80.7	4.70	0.015
60	0.67240	-2.384	0.6565		-4.342	0.35840	0.9565	7	-32.894	81.1	5.13	0.012
26	0.92140	-2.285			-4.458	0.00351		5	-35.395	81.5	5.50	0.010
34	0.06091	-2.156					0.9359	4	-36.567	81.6	5.61	0.009
36	0.12020	-2.085	0.6121				1.0570	5	-35.493	81.7	5.70	0.009
6	1.03200	-1.925		-2.475				4	-36.656	81.8	5.79	0.009
63	2.49400		0.9392	-8.704	-3.918	6.07600	1.9600	7	-33.275	81.9	5.89	0.008
28	0.91010	-2.258	0.5314		-4.374	0.41190		6	-34.653	82.3	6.30	0.007
22	1.04800	-1.804		-4.131		2.22600		5	-35.805	82.3	6.32	0.007
55	1.77300		0.9481	-7.708		4.80500	2.1500	6	-34.684	82.3	6.36	0.006
61	2.68700			-9.479	-4.177	6.41100	1.8880	6	-35.104	83.2	7.21	0.004
8	1.03300	-1.880	0.3544	-2.317				5	-36.295	83.3	7.30	0.004
24	1.03800	-1.717	0.4715	-4.029		2.41400		6	-35.189	83.4	7.38	0.004
2	0.21820	-2.023						3	-38.695	83.7	7.68	0.003
50	0.10170	-2.149				-0.16970	0.9415	5	-36.556	83.8	7.82	0.003
52	0.02703	-2.105	0.6577			0.38550	1.0630	6	-35.439	83.9	7.87	0.003
53	1.84100			-8.371		5.05800	2.0430	5	-36.601	83.9	7.91	0.003
4	0.28800	-2.007	0.4210					4	-38.090	84.6	8.66	0.002
18	0.23530	-2.023				-0.07180		4	-38.692	85.8	9.86	0.001
47	1.84300		0.7727	-4.098	-3.433		1.9390	6	-36.692	86.4	10.38	0.001
39	1.37100		0.7590	-4.042			1.9570	5	-38.014	86.7	10.74	0.001
20	0.24320	-2.008	0.4374			0.19640		5	-38.071	86.8	10.85	0.001
45	1.78600			-4.316	-3.453		1.8030	5	-38.252	87.2	11.21	0.001
37	1.30700			-4.270			1.8190	4	-39.585	87.6	11.65	0.000
31	1.87900		0.6620	-5.940	-3.523	4.04900		6	-37.512	88.0	12.02	0.000
29	1.91800			-6.471	-3.466	4.32500		5	-38.982	88.7	12.67	0.000

```
23 1.25700        0.5661  -5.440          3.25400         5 -40.133  91.0 14.98  0.000
21 1.26100                -5.798          3.41400         4 -41.342  91.1 15.16  0.000
15 1.59100        0.5289  -2.996  -2.814                  5 -40.866  92.4 16.44  0.000
13 1.55400                -3.184  -2.782                  4 -41.985  92.4 16.45  0.000
5  1.10900                -3.268                          3 -43.787  93.8 17.86  0.000
7  1.14400        0.4718  -3.114                          4 -42.850  94.2 18.18  0.000
43 0.43640        0.7854          -2.267          1.1660  5 -43.714  98.1 22.14  0.000
35 0.10790        0.7764                          1.2850  4 -44.904  98.3 22.28  0.000
59 0.43900        0.7843          -2.269 -0.01168 1.1660  6 -43.714 100.4 24.43  0.000
51 0.11810        0.7721                 -0.04586 1.2850  5 -44.902 100.5 24.51  0.000
41 0.39030                        -2.288          0.9278  4 -46.312 101.1 25.10  0.000
33 0.05802                                        1.0270  3 -47.519 101.3 25.33  0.000
11 0.58270        0.5537          -2.364                  4 -47.065 102.6 26.60  0.000
57 0.49420                        -2.362 -0.47570 0.9858  5 -46.164 103.0 27.04  0.000
49 0.15860                               -0.47620 1.0630  4 -47.362 103.2 27.20  0.000
9  0.52570                        -2.351                  3 -48.616 103.5 27.52  0.000
3  0.18670        0.5104                                  3 -49.190 104.6 28.67  0.000
27 0.58570        0.5528          -2.364 -0.01391         5 -47.064 104.8 28.84  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Appendix VI

Table VII.5 – All models (up to the first 60), for the hypotheses of disturbance, landcover composition and hybrid, tested for the distribution of stone marten . The degrees of freedom (df) and the Akaike's Information Criterion for small samples (AICc) are presented. LogLik represents the models' log-likelihood. Within each hypothesis, the models are ranked by the ΔAICc value (delta) – variation between the AICc of each model and the lower AICc detected for models in the same hypothesis. The probability of each model being the best in each hypothesis is presented through the Akaike weight (weight). All the v ariables used to test the different ecological hypotheses are described in Table IV.1. Intercept is represented by (Int).

Disturbance hypothesis (H1)

```
Global model call: glmer(formula = INDICIO ~ Pollution + Debris + Livestock + Fire + Infra + Machinery +
        + Vehicles + Dist_urban + (1 | CONCELHO), data = matrix_fuinha, family = binomial)
---
Model selection table
       (Int) Pollution  Debris  Livestock Fire   Infra  Machinery  Vehicles Dist_urban df logLik AICc  delta weight
205  0.3197000                    -2.041  -15.63                        -15.65 -0.4893  6 -70.884 154.4  0.00  0.074
77   0.3705000                    -1.884  -18.45                        -17.32          5 -72.241 154.9  0.53  0.057
141  0.2648000                    -2.011  -14.94                                -0.5246  5 -72.254 155.0  0.55  0.056
206  0.3160000  1.4870            -2.165  -16.07                        -16.51 -0.4900  7 -70.207 155.3  0.87  0.048
78   0.3647000  1.5240            -2.005  -18.69                        -18.20          6 -71.540 155.7  1.31  0.038
221  0.3968000                    -2.078  -15.59 -0.7230                -15.97 -0.5417  7 -70.441 155.7  1.33  0.038
13   0.3144000                    -1.836  -16.72                                        4 -73.819 155.9  1.53  0.034
157  0.3382000                    -2.048  -29.16 -0.7038                        -0.5769  6 -71.841 156.3  1.91  0.028
207  0.3449000         -0.39260   -2.019  -16.92                        -16.86 -0.4836  7 -70.803 156.5  2.06  0.026
222  0.3971000  1.5960            -2.201  -15.64 -0.7666                -16.51 -0.5451  8 -69.703 156.5  2.11  0.026
237  0.3363000                    -2.022  -15.97         -0.114900      -15.70 -0.4981  7 -70.864 156.6  2.18  0.025
142  0.2496000  0.8452            -2.074  -14.45                                -0.5349  6 -71.982 156.6  2.20  0.025
93   0.4231000                    -1.893  -18.15 -0.4511                -17.63          6 -72.039 156.7  2.31  0.023
143  0.3060000         -0.58380   -1.991  -15.78                                -0.5156  6 -72.063 156.8  2.36  0.023
79   0.3969000         -0.41440   -1.869  -18.31                        -17.44          6 -72.148 156.9  2.53  0.021
109  0.3649000                    -1.890  -17.72          0.035460      -18.04          6 -72.239 157.1  2.71  0.019
173  0.2697000                    -2.005  -14.23         -0.035600              -0.5272  6 -72.252 157.1  2.74  0.019
208  0.3458000  1.5520 -0.48640   -2.143  -15.48                        -16.74 -0.4838  8 -70.081 157.3  2.87  0.018
94   0.4224000  1.6030            -2.016  -18.16 -0.4999                -18.24          7 -71.291 157.4  3.03  0.016
```

238	0.3381000	1.5100		-2.140	-15.91		-0.150700	-16.57	-0.5007	8	-70.172	157.5	3.05	0.016
14	0.2994000	0.7889		-1.885	-16.94					5	-73.585	157.6	3.22	0.015
15	0.3596000		-0.63020	-1.817	-17.36					5	-73.590	157.6	3.23	0.015
80	0.3958000	1.5940	-0.51190	-1.989	-18.76			-17.82		7	-71.397	157.7	3.25	0.015
29	0.3602000			-1.845	-16.86	-0.4051				5	-73.657	157.8	3.36	0.014
223	0.4309000		-0.46990	-2.057	-16.77	-0.7475		-16.57	-0.5365	8	-70.326	157.8	3.36	0.014
110	0.3657000	1.5250		-2.004	-18.42		-0.006173	-18.28		7	-71.540	157.9	3.53	0.013
253	0.4127000			-2.060	-15.54	-0.7202	-0.109600	-15.45	-0.5499	8	-70.422	158.0	3.55	0.012
158	0.3227000	0.8679		-2.108	-28.82	-0.7117			-0.5878	7	-71.561	158.0	3.57	0.012
45	0.2968000			-1.858	-17.03		0.116400			5	-73.795	158.0	3.63	0.012
159	0.3908000		-0.66120	-2.034	-16.01	-0.7428			-0.5700	7	-71.600	158.1	3.65	0.012
144	0.3002000	1.0480	-0.75770	-2.054	-14.86				-0.5231	7	-71.673	158.2	3.80	0.011
224	0.4408000	1.7090	-0.60310	-2.185	-16.47	-0.8134		-18.10	-0.5408	9	-69.513	158.4	4.02	0.010
189	0.3422000			-2.043	-14.32	-0.7025	-0.029840		-0.5791	7	-71.840	158.5	4.13	0.009
239	0.3640000		-0.40320	-1.998	-16.82		-0.125900	-16.91	-0.4929	8	-70.778	158.7	4.26	0.009
95	0.4567000		-0.46810	-1.880	-18.26	-0.4762		-17.56		7	-71.922	158.7	4.29	0.009
254	0.4166000	1.6110		-2.178	-15.49	-0.7590	-0.134500	-17.00	-0.5541	9	-69.674	158.8	4.35	0.008
174	0.2547000	0.8458		-2.067	-14.68		-0.037220		-0.5375	7	-71.980	158.8	4.41	0.008
125	0.4158000			-1.902	-18.05	-0.4539	0.047370	-17.50		7	-72.035	158.9	4.52	0.008
175	0.3157000		-0.59130	-1.971	-15.80		-0.059310		-0.5176	7	-72.057	159.0	4.57	0.008
16	0.3521000	1.0090	-0.80200	-1.875	-16.89					6	-73.234	159.1	4.70	0.007
111	0.3931000		-0.41210	-1.873	-17.90		0.022520	-17.10		7	-72.147	159.2	4.75	0.007
197	0.0714600			-2.061				-13.55	-0.7299	5	-74.411	159.3	4.87	0.006
96	0.4653000	1.7090	-0.59740	-2.004	-18.40	-0.5458		-17.62		8	-71.100	159.3	4.91	0.006
31	0.4149000		-0.68260	-1.829	-17.50	-0.4456				6	-73.392	159.4	5.02	0.006
160	0.3887000	1.1320	-0.86890	-2.098	-14.68	-0.7755			-0.5798	8	-71.168	159.5	5.04	0.006
240	0.3733000	1.5870	-0.51210	-2.114	-15.65		-0.174800	-16.74	-0.4955	9	-70.034	159.5	5.06	0.006
30	0.3450000	0.8007		-1.891	-16.98	-0.4097				6	-73.420	159.5	5.07	0.006
133	0.0295000			-2.031					-0.7507	4	-75.598	159.5	5.09	0.006
126	0.4205000	1.6000		-2.018	-18.19	-0.5009	0.012480	-18.44		8	-71.291	159.7	5.29	0.005
46	0.2823000	0.7845		-1.908	-17.04		0.114200			6	-73.562	159.8	5.36	0.005
47	0.3451000		-0.61780	-1.833	-17.31		0.088000			6	-73.576	159.8	5.38	0.005
61	0.3414000			-1.869	-16.92	-0.4131	0.127300			6	-73.628	159.9	5.49	0.005
112	0.4017000	1.6040	-0.51790	-1.984	-17.62		-0.033990	-18.47		8	-71.395	159.9	5.50	0.005
198	0.0707500	1.6100		-2.196				-14.48	-0.7264	6	-73.660	160.0	5.55	0.005
255	0.4497000		-0.48010	-2.036	-16.78	-0.7443	-0.121900	-16.71	-0.5453	9	-70.302	160.0	5.60	0.004
190	0.3262000	0.8676		-2.103	-30.57	-0.7104	-0.026050		-0.5895	8	-71.560	160.2	5.83	0.004
191	0.4000000		-0.66770	-2.013	-15.55	-0.7389	-0.055750		-0.5707	8	-71.595	160.3	5.90	0.004
176	0.3116000	1.0570	-0.76950	-2.040	-14.64		-0.079040		-0.5288	8	-71.663	160.4	6.03	0.004

```
256  0.4664000  1.7370 -0.62690  -2.159  -16.82 -0.8060 -0.162200      -17.54 -0.5514 10 -69.471 160.7  6.27  0.003
134  0.0164600  1.0380           -2.110                                -0.7540  5 -75.205 160.9  6.45  0.003
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Landcover composition hypothesis (H3)

```
Global model call: glmer(formula = INDICIO ~ Water + Agr_area + Forest + Shrub + Spars_veg +
    (1 | CONCELHO), data = matrix_fuinha, family = binomial)
---
Model selection table
     (Int) Water Agr_area Forest Shrub Spars_veg df logLik  AICc delta weight
18 -3.5860 3.647                          -28.89  4 -82.632 173.6  0.00  0.105
20 -3.6910 3.443 0.2858                   -29.60  5 -81.785 174.0  0.46  0.083
2  -0.1633 3.934                                  3 -83.932 174.0  0.48  0.083
4  -0.2358 3.291 0.2856                           4 -83.109 174.5  0.95  0.065
19 -4.0570       0.3279                   -29.74  4 -83.400 175.1  1.54  0.049
17 -4.0060                                -29.36  3 -84.606 175.4  1.83  0.042
26 -3.6210 3.723                  0.1001  -29.31  5 -82.526 175.5  1.94  0.040
32 -4.0330 3.930 0.6210  0.684900 0.6405  -32.40  7 -80.404 175.7  2.10  0.037
3  -0.5665       0.3301                         3 -84.755 175.7  2.12  0.036
22 -3.6190 3.695        -0.004966         -29.22  5 -82.632 175.7  2.15  0.036
28 -3.7270 3.446 0.3081           0.1462  -29.95  6 -81.565 175.8  2.21  0.035
1  -0.5602                                        2 -85.945 176.0  2.41  0.031
24 -3.7020 3.751 0.3162  0.103200         -29.95  6 -81.695 176.0  2.47  0.031
10 -0.2022 3.565                  0.0782         4 -83.868 176.0  2.47  0.030
6  -0.2012 3.605         0.007795                4 -83.931 176.2  2.60  0.029
16 -0.3333 3.143 0.6534  0.740300 0.6639         6 -81.770 176.2  2.62  0.028
12 -0.2111 3.482 0.3038           0.1220         5 -82.959 176.4  2.81  0.026
8  -0.2539 3.179 0.3232  0.122000                5 -82.988 176.4  2.86  0.025
27 -4.0710       0.3528           0.1664  -29.90  5 -83.112 176.7  3.11  0.022
31 -4.4550       0.6027  0.570900 0.5730  -32.70  6 -82.191 177.0  3.46  0.019
23 -4.0540       0.3466  0.066240         -29.70  5 -83.362 177.2  3.61  0.017
25 -4.0150                        0.1117  -29.48  4 -84.471 177.2  3.68  0.017
11 -0.5648       0.3510           0.1426         4 -84.548 177.4  3.83  0.015
21 -4.0110              -0.053240         -29.41  4 -84.578 177.5  3.89  0.015
```

```
30 -3.6270 3.920         0.107900 0.1652 -29.54  6 -82.461 177.6  4.00  0.014
15 -0.6228       0.6246  0.605000 0.5793         5 -83.617 177.7  4.12  0.013
7  -0.5692       0.3548  0.083770                4 -84.696 177.7  4.13  0.013
9  -0.5570                       0.0900         3 -85.860 177.9  4.33  0.012
5  -0.5596              -0.042850                3 -85.928 178.0  4.47  0.011
14 -0.2039 3.565         0.105800 0.1426         5 -83.808 178.1  4.51  0.011
29 -4.0140               0.035810 0.1330 -29.48  5 -84.463 179.4  5.82  0.006
13 -0.5570               0.029360 0.1076         4 -85.855 180.0  6.45  0.004
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
```

Hybrid hypothesis (H4)

```
Global model call: glmer(formula = INDICIO ~ Agr_area + Livestock + Dist_urban + Shrub +
   + (1 | CONCELHO), data = matrix_fuinha, family = binomial)
---
Model selection table
    (Int) Agr_area Livestock Dist_urban Shrub df logLik AICc delta weight
8   0.045300 0.3594  -2.093 -0.6717          5 -74.491 159.4  0.00  0.321
7   0.029500         -2.031 -0.7507          4 -75.598 159.5  0.06  0.311
16  0.042570 0.3760  -2.081 -0.6887 0.16520  6 -74.265 161.2  1.73  0.135
15  0.030140         -2.025 -0.7661 0.12680  5 -75.460 161.4  1.94  0.122
4   0.041330 0.4618  -1.865                 4 -77.285 162.9  3.43  0.058
12  0.043870 0.4744  -1.851         0.12720  5 -77.144 164.7  5.31  0.023
3   0.003948         -1.746                 3 -79.309 164.8  5.36  0.022
11  0.007168         -1.740         0.07415  4 -79.259 166.8  7.38  0.008
5  -0.618600                -0.5147          3 -83.854 173.9 14.45  0.000
6  -0.623900 0.2514         -0.4569          4 -83.220 174.7 15.30  0.000
13 -0.620000                -0.5340 0.13810  4 -83.664 175.6 16.19  0.000
2  -0.566500 0.3301                         3 -84.755 175.7 16.25  0.000
1  -0.560200                               2 -85.945 176.0 16.54  0.000
14 -0.629600 0.2776         -0.4781 0.17850  5 -82.913 176.3 16.84  0.000
10 -0.564800 0.3510                 0.14260  4 -84.548 177.4 17.96  0.000
9  -0.557000                 0.09000  3 -85.860 177.9 18.46  0.000
Models ranked by AICc(x)
Random terms (all models): '1 | CONCELHO'
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